STUDIES ON HALO BLIGHT, A BACTERIAL DISEASE OF OATS

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A THESIS
submitted to the University of Alberta
in partial fulfilment of the
requirements for the degree of
MASTER OF SCIENCE

Edmonton, Alberta
April, 1943

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

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INTRODUCTION

The damage from bacterial diseases of oats is probably far greater than is generally realized. Such diseases no doubt will assume more importance in Canada as the oat acreage increases. The demand for livestock and the wheat reduction program are both encouraging greater production of the coarse grains.

Only a few bacterial pathogens of oats have been described and the literature regarding them is not extensive. Two have been reported to occur naturally on the oat plant: *Bacterium coronafaciens* Elliott, the causal organism of Halo Blight of oats; and *Bacterium striafaciens* Elliott, the causal organism of Bacterial Stripe Blight of oats.

Bacterium alboprecipitans Rosen, *B. coronafaciens atropurpureum* Reddy and Godkin, *Xanthomonas translucens* f. sp. *hordeiavenae* f. sp. nov. Hagborg, and *X. translucens* f. sp. *cerealis* f. sp. nov. Hagborg have also been reported to be pathogenic to oats, but only under artificial conditions.

THE HALO BLIGHT DISEASE OF OATS

The Halo Blight disease of oats is widespread in western Canada, especially when the season is favorable. All varieties of cultivated oats appear to be naturally susceptible in varying degrees. In addition other crops such as wheat, rye, and barley may be slightly susceptible under artificial conditions.

The causal organism, B. coronafaciens, is able to remain alive for some years in the seed and for some time in or on diseased plant residues. Seed-borne inoculum is most likely to cause primary infection of the emerging coleoptile and the first leaves of the oat seedling, especially during wet, cool and cloudy weather in the late spring. Higher temperatures and less moisture tend to reduce the severity of the disease, and the damage under such conditions may be negligible.

Inoculum from the primary lesions on the first seedling leaves may be carried to the other leaves by wind and rain, causing secondary infections. According to Elliott (15) the lesions from these secondary infections first appear on the leaf as light green spots, sunken in the middle. These enlarge rapidly, showing concentric rings in various shades of yellow, green, and brown. Later on the lesions become dry and yellowish brown.(Figure 1). At no time is there any bacterial exudate. The spikelets, too, are often

Figure 1

Leaves of Erban oats affected with Halo Blight, showing the typical haloed lesions of secondary infections, as seen in the field

attacked. The typical halo may appear on the chaff, and the tissues between the veins may turn yellow and later translucent. The pedicels dry and shrink, and the receptacles become shiny and translucent, giving the panicle a blighted appearance (Figure 2). In severely affected crops, these characteristic panicles are easily recognized. In less severely affected ones, only the lower leaves may be dead, while the newer growth may be entirely free of lesions. Lesions on the flag leaf are often in a favorable position to supply inoculum to the emerging panicle. This may cause partial or complete blighting of the spikelets, The grains may then be blighted or contaminated by the organism. Sometimes they may show no outward symptoms of the disease, yet may carry sufficient inoculum to initiate primary infection.

Bacterial Stripe Blight caused by _B. striafaciens_ can be distinguished from Halo Blight by the thin white scales of bacterial exudate and by the absence of the halo (16).

Inoculum from contaminated seed is probably the most important to be contended with and is the cause of much emergence blight. Under the unfavorable weather conditions noted before, the secondary inoculum from the first leaf may not cause much infection and the later growth may be entirely free of disease. But under suitable conditions this inoculum may be widely spread and cause severe damage to the later leaves and heads. Thus the disease can be prevented if the

Figure 2

Panicles of Erban oats naturally affected with
Halo Blight, in the field, showing
the shrunken spikelets

pathogen is controlled while on or in the seed. Since a few infected plants, under suitable conditions, can furnish initial inoculum for a widespread distribution of the pathogen, the control must be complete to be effective.

The determination of the effect of seed treatment on the control of Halo Blight in the field is often difficult under favorable conditions for the spread of the inoculum. For example, it is a common observation under Edmonton field conditions that oat plantings sown from treated seed differ little in severity of Halo Blight from those started with untreated seed. This may occur as a result of the rapid development of secondary infections produced by inoculum originating in untreated seed or in inefficiently treated seed.

The evaluation of seed treatment for the control of primary infection can be more readily studied under controlled greenhouse conditions, since secondary infections can be largely avoided there. The present studies were made under such conditions.

OBJECTS OF THE STUDIES

The physiological reactions of *B. coronafaciens* have been carefully worked out by Miss Elliott (15). The present studies deal particularly with primary infection and measures designed to prevent it by the destruction of seed-borne

inoculum. The principal objects were to determine:

1. The identity of isolates from a field of blighted oats as B. coronafaciens;

2. The effect of chemical treatment on artificially inoculated seed;

3. The effect of environmental conditions on primary infection;

4. The extent of antibiotic effects of other microorganisms toward B. coronafaciens in artificial media and in soil;

5. The effect of chemical seed treatment on the oat host plant and on other cereals for comparative purposes.

THE CAUSAL ORGANISM

Source of Isolates

In the summer of 1941, severe Halo Blight was noted in a field of Erban oats at Morningside, Alberta. Specimens of affected leaves and panicles were secured. In addition collections were made at Edmonton and Fallis. These included typical haloed lesions on the leaf (Figure 1) and blighted spikelets (Figure 2). The latter were easily distinguishable in the field from spikelets affected with physiological blast by the discolored, smooth, shiny appearance of the spikelet, and the shrunken pedicel. In addition, the flag leaf invariably possessed lesions from which the inoculum infected the emerging panicle.

Methods of Isolation

The organism was readily isolated from the diseased tissue, even after eight months' storage at room temperature. Portions of this tissue were put through six successive washings with sterile distilled water in petri dishes. These were then crushed in sterile water blanks and, from the suspension, loop dilutions were plated out in 2% dextrose agar.

Two organisms predominated. A viscid yellow one was commonly present. This was believed to be similar to the Bacillus avenae of Manns who considered it to be necessary before the white one could be actively pathogenic. Miss Elliott (15) however believes it to be merely a surface saprophyte. In our tests there were no indications that this organism was necessary for the white one to become pathogenic.

Pathogenicity tests by leaf inoculation

The isolates were tested for pathogenicity by inoculating the leaves of Victory oat seedlings without injury, using inoculum direct from the culture tubes. The plants were then placed in a humidity chamber for 24 hours and then returned to the greenhouse bench. The production of lesions was taken as an index of pathogenicity.

The typical field lesions as illustrated in Figure 1 were never obtained. The usual reaction was a light brown

region bounded by a more or less circular reddish brown ring and the whole by a yellowish region. Often the ring was absent, giving the lesion the appearance of a large circular to oval chlorotic area with normal turgidity. Often long water-soaked streaks developed along the leaf, similar to the lesions of Stripe Blight. These probably arose as a result of the organism's being carried down the leaf by drops of water of guttation.

Inoculation of oat leaves, water-soaked with a five-minute exposure to a water jet connected to an air line at 30 pounds pressure, produced very severe symptoms which resulted in the quick shrivelling and death of the leaf. This was considered comparable to the "epidemic type" of lesion described by Clayton (10) as resulting from inoculation of water-soaked tobacco leaves with B. tabacum. The uninoculated water-soaked leaves soon recovered their normal appearance. Thus water-soaking in the field may be the reason for the severe damage often done to the lower leaves of affected oat plants.

Isolates selected for study were T14, T31, T33b, T37, and T47.

Cultural Characteristics

The procedures laid down in the Manual of Methods for Pure Culture Study of Bacteria were generally followed except where otherwise noted.

Cell morphology and Gram reaction

On potato dextrose agar the organism is a rod .59 to .85 x 1.77 to 2.95 microns. On nutrient agar and tryptone-glucose skim-milk agar the rods tend to occur in chains. The measurements were made on smears stained with nigrosin. Capsules were demonstrated by Anthony's method. No spores were observed with the use of Dorner's method. The Gram reaction was negative.

Temperature relations

The organism grew well on potato dextrose agar at temperatures from 7°C to 30°C (Figure 4). At 5°C and 35°C no growth took place. Plates incubated at the latter temperatures showed no growth when transferred to room temperature of 24°C after twelve days.

Growth on media

Growth was best on 2% potato dextrose agar. On plates the surface colonies are white, raised, circular with entire margins, and of a starchy consistency; the sub-surface colonies are lens shaped. Under the low power the colonies have a granular appearance. In potato dextrose agar the organism has a characteristic sour odor.

On tryptone-glucose skim-milk agar the growth is colorless and translucent with undulate margins. In nutrient

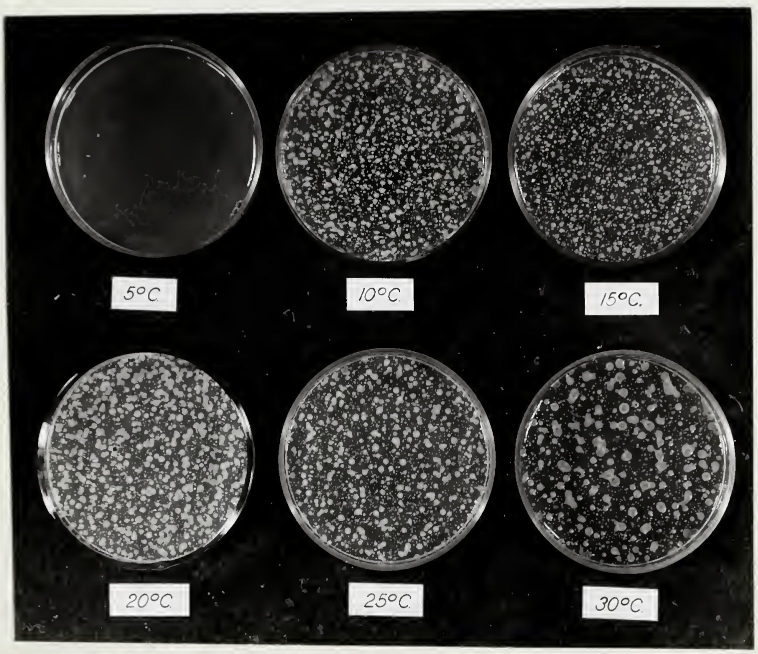

Figure 3

Growth of B. coronafaciens on potato dextrose agar at different temperatures. No growth occurred at 5°C. or 35°C.

agar, the growth is sparse and white.

Chromogenesis

A green, water-soluble pigment appeared in three to four days in Sullivan's modified asparagin medium (9). This was accompanied by the formation of unidentified white crystals. Pigment production appeared to be associated with pathogenicity.

On tryptone-glucose skim-milk agar, the organism caused a noticeable greening of the medium. This effect was not noted on potato dextrose agar or nutrient agar. In ultraviolet light no fluorescence was produced by the colonies on any of these media.

Physiological reactions

The Gnezda test for indol production in tryptone was negative. The Methyl-red test in peptone-glucose-K_2HPO_4 showed non-production of acid in this medium. The Voges-Proskauer test for the production of acetyl-methyl-carbinol was also negative.

Slight growth on Simmon's citrate medium indicated some utilization of citrate carbon, but the reaction of the medium was unchanged. No nitrate reduction in .1% KNO_3 nutrient broth or hydrolysis of starch on .2% soluble starch nutrient agar was noted. In litmus milk, the reaction was alkaline and there was no curdling. Gelatin in nutrient broth

was slowly liquefied at 21°C.

Acid was formed from dextrose and fructose but not from salicin. No gas was formed in Durham tubes.

Summary

The description, based on the above tests, agrees in general with that of Miss Elliott (17) and is as follows:

Gram negative, capsule-forming, non-sporing rod, .59 to .85 x 1.77 to 2.95 microns, flourescin produced in asparagin medium, gelatin liquefied, citrate carbon utilized, indol negative, methyl red negative, acetyl-methyl-carbinol negative, nitrate reduction negative, starch hydrolysis negative, no gas formed, acid formed in dextrose and fructose, no acid in salicin, alkali formed in litmus milk, temperature range 30°C to 7[illegible] $^{\circ}$C.

On the basis of the cultural characteristics and the symptoms caused by secondary inoculation, the identity of the isolates was determined to be B. coronafaciens. This was substantiated by comparison with a culture of this organism from Dr. W. A. F. Hagborg of Winnipeg, also pathogenic on oat seedlings. A culture of B. striafaciens from the same source was considered to bear no resemblance to our isolates.

The original name Bacterium coronafaciens given by Miss Elliott is used throughout these studies. The name Pseudomonas coronafaciens (Ell.) Stapp is probably preferable now in view of Dowson's new classification (13), but until the latter is more widely adopted, the original name is retained.

The varietal host range

Elliott (15) first observed varietal reactions to B. coronafaciens. She noted that all varieties examined were susceptible and that differences between varieties became less noticeable in severe attacks as the season advanced. Aamodt and Platt (1) also failed to find immunity in any of the varieties tested in a year of severe infection, though under other conditions some differences in susceptibility appeared to be exhibited.

A number of varieties obtained from the Cereal Division, University of Alberta, were inoculated hypodermically at the seedling stage in the greenhouse with a water-suspension of B. coronafaciens T47, incubated , and later compared. Differences in reaction were noted, but all the varieties proved susceptible to a greater or lesser extent. The ratings were based on the extent of the chlorotic areas. The results are shown in Table I.

TABLE I

The comparative reaction of thirty oat varieties to B. coronafaciens

Variety	Rating	Variety	Rating
A. fatua glabrata	+++	Legacy	++
Roxton	+++	Nakota	++
Victory	+++	Ripon	++
Abundance	++	Tama	++
Avena brevis	++	Valor	++
Banner	++	Vanguard	++
Brighton	++	White Cross	++
Dasix	++	Ajax	+
Eagle	++	Alaska	+
Early Miller	++	Anthony	+
Erban	++	Avena fatua	+
Gopher	++	Exeter	+
Kanota	++	Markton	+
Laurel	++	Nidar	+
Leader	++	Simo	+

STUDIES ON SEED TREATMENT FOR THE CONTROL OF PRIMARY INFECTION

Introduction

Seed treatment was investigated as a control measure for Halo Blight by Elliott in 1919. Two methods were used: (1) soaking the seed in 1:320 formaldehyde for three hours; and (2) subjecting it to hot air at 100°C for 30 hours. The former killed many but not all of the bacteria on the seed, while the latter was completely effective in control. Neither treatment, however, can be considered satisfactory--the hot

air treatment because of its impracticability and the formaldehyde dip because of the trouble involved and possible injury to the seed.

A series of experiments was made to determine the relative effectiveness of seed treatment with Ceresan, Spergon, and formaldehyde in the control of Halo Blight. Ceresan and Spergon are the trade names for dust preparations containing ethyl mercury phosphate and tetrachloro-para-benzoquinone respectively. The formaldehyde treatment consisted of a wet application of a 1:320 solution of commercial formalin (Appendix XII). Since naturally infested seed was not available, seed artificially inoculated with isolates from diseased tissue was used.

Method of Seed Inoculation

In these studies a heavy seed inoculation was considered desirable for comparing the effects of different seed treatments. It was thought especially important to deposit some of the inoculum between the hull and the caryopsis. This was done by washing the seed in six to eight changes of sterile water under a partial vacuum, soaking it for about half an hour in a water suspension of the bacteria, and again subjecting it to a partial vacuum. The seed was then dried at room temperature before the chemical treatments were made.

The Symptoms of Primary Infection

Miss Elliott described the primary lesion development in the field as follows: "Lesions are first visible as light green oval spots 4 to 5 mm. in diameter with central sunken points of infection at first evident only on one side of the leaf. This center of infection increases slowly in size, penetrates the leaf tissue, and in a day or two forms a grey or brown dry tissue from one to several millimetres in diameter, evident on both sides of the leaf blade. The halo-like margin spreads rapidly becoming uniformly lighter green to yellow or showing concentric markings of different shades of green and yellow"(15).

Under the conditions of these experiments, infected plants, when two to three inches in height, usually showed translucent, water-soaked regions between the veins when the humidity was high, usually in the mornings and evenings. Later in the day as the humidity decreased, these areas disappeared and the leaf became quite dry and normal in appearance. This sequence was repeated for three to four days or longer, and the leaf gradually became chlorotic, ultimately shrivelling or collapsing (Figure 4). At other times, dark, shrunken areas appeared, usually at the tips or along the edges of the blade. Chlorotic areas developed around these and rapidly enlarged. Often a distinct streak of shrunken tissue extended along the whole length of the blade, probably

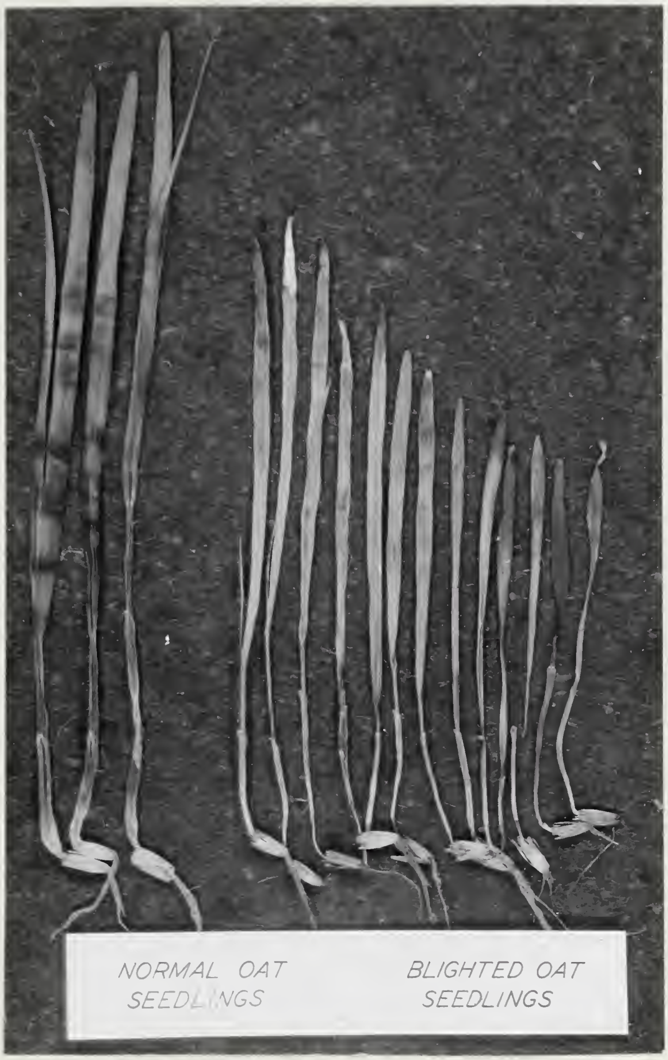

Figure 4

Blighted oat seedlings from seed inoculated with B. coronafaciens compared with normal three-week-old seedlings

due to inoculum flowing down in the water of guttation. Less frequently reddish brown lesions with or without the chlorotic areas occurred, as noted by Hagborg (25). The latter type was also common when needle inoculations were made on leaves water-soaked for five minutes with a fine spray of water under thirty pounds air pressure.

Effect of Seed Treatment with Formaldehyde and Ceresan on hulled* and hulless oats inoculated with _B. coronafaciens_

This experiment was made to compare the effects of Ceresan treatment with the standard formaldehyde treatment on hulled and hulless oats inoculated with isolates of _B. coronafaciens_ from blighted oats.

Method

Hulled and hulless Erban oats were used, the latter being dehulled by hand. After six 5-minute washings with sterile water under a partial vacuum, the seed was inoculated as previously described with water-suspensions of cultures T33b and T37, with water as a control. The seed was left in

* By hulled oats is meant oats with hull, while hulless oats refers to oats without the hull.

this inoculum for three hours, drained, and left overnight to dry. Separate lots were then treated. The Ceresan was applied by shaking the dust and grain together in a 200 cc. Erlenmeyer flask. It was used at the recommended rate of 1/2 oz. per bushel. After treatment the flasks were bunged with cotton and left for 12 hours. The formaldehyde was applied by immersing the seed in a 1:320 solution for two minutes, draining, covering with cheesecloth for four hours, and then drying at room temperature.

The seeds were planted in glass-fronted galvanized iron cans 22 x 12 x 1.75 inches in size, kindly made available by the Cereal Division. The joints were sealed with battery sealing compound to make the cans water tight.

The cans contained 7800 grams of finely sifted 5:1 Edmonton soil and sand mixture, having a pH of 6.2, and adjusted to a moisture content of 17.6%. Twenty-five kernels were sown in each container, one inch below the surface of the soil, embryo pointing downwards, and against the glass. After seeding each was watered with 1000 cc. of tap water and placed at an angle of 45 degrees in specially constructed frames. This allowed the roots to grow against the glass so that daily observations of their development could be made.

The glass was marked off into one-inch divisions from the seed level to the bottom of the glass. The number of root tips within each division as counted, multiplied by the lengthto the closest inch gave an estimate of the root

growth of the seedlings growing in the container.

Some difficulty was experienced from roots not growing their whole length along the glass. This may account for some of the anomalies attributed to observer error. However, these were generally ironed out on averaging. Examination of the root systems by removal of the glass front showed that they all grew close to or against the glass, and this method may therefore be considered to give a fairly good estimate of the root lengths.

Results

Data on emergence, leaf length, and root growth, taken on the fourteenth day after seeding are summarized in Table II; the results of the analysis of variance in Table III; the effect of inoculation in general on hulled and hulless oats in Table IV; the effect of seed treatments in general on hulled and hulless oats in Table V; the daily root growth for twelve days in Appendix I; and a graphical comparison of the daily root growth from inoculated and non-inoculated seed is shown in Figure 5.

Culture T33b was effective in lowering emergence, leaf length, and root length of plants from both hulless and hulled seed, while culture T37 caused a significant decrease only in leaf length of hulless oats.

No significant differences were shown by the treatments with the exception of the formaldehyde on hulless oats,

TABLE II

Effect of seed treatment on hulled and hulless oats inoculated with B. coronafaciens

Culture	Hull	Treatment	Emergence (%)	% emergence converted to degrees*	Leaf length (inches) (14th day)	Root length (inches) (14th day)
T33b	Hulled	Formaldehyde 1:320	81.0	65.1	184.3	414.8
		Ceresan ½ oz.	77.0	61.4	181.3	400.5
		Check	77.0	61.7	168.5	383.3
	Hulless	Formaldehyde 1:320	10.0	17.6	13.0	49.5
		Ceresan ½ oz.	29.0	32.5	37.0	87.5
		Check	46.0	42.7	84.5	199.3
T37	Hulled	Formaldehyde 1:320	92.0	75.8	199.8	436.5
		Ceresan ½ oz.	100.0	90.0	226.8	421.8
		Check	97.0	83.0	221.8	451.0
	Hulless	Formaldehyde 1:320	31.5	34.0	46.3	132.8
		Ceresan ½ oz.	64.0	53.2	108.8	240.3
		Check	63.0	52.6	132.8	313.3
Control	Hulled	Formaldehyde 1:320	96.0	81.8	222.8	450.3
		Ceresan ½ oz.	98.0	84.3	230.5	489.3
		Check	96.0	80.2	231.3	503.8
	Hulless	Formaldehyde 1:320	32.0	33.8	50.3	134.0
		Ceresan ½ oz.	79.0	62.8	124.5	232.8
		Check	78.0	62.4	191.5	360.5
Minimum significant difference - treatments in general			10.5	16.0	35.4	80.1

* $p = \sin^2\theta$

TABLE III

Analysis of variance for seed treatment on hulled and hulless oats inoculated with <u>B. coronafaciens</u>

Variance due to	D.F.	Mean square: Germination (%)	Converted germination to $\sin^2 \theta$	Leaf length (inches)	Root length (inches)
Inoculations	2	4663**	3031**	25367**	76318**
Replicates	3	26	10	125	5137
Error (1)	6	58	34	802	4947
Hull	1	32513**	18912**	258600**	1103107**
Hull x Inoc.	2	421**	81	1163	9454
Error (2)	9	46	55	462	2623
Treatments	2	2633	1266	16870	64630
Treat. x Hull	2	2447**	871**	13421**	29436**
Treat. x Inoc.	4	177*	84	1421	4605
Treat. x Inoc. x Hull	4	109	80	675	7079
Error (3)	36	59	42	655	3192
Total	71				

* Significant to the 5% point
** Significant to the 1% point

TABLE IV

Summary of the effect of inoculation, from the data in Table II

Organism	Emergence (%)	Converted emergence	Leaf length	Root length
Hulled oats				
T33b	78.7*	62.7	178.0*	399.5*
T37	96.3	82.9	216.2	436.4
Control	96.7	82.1	228.2	489.4
Hulless oats				
T33b	28.3*	30.9*	44.8*	112.0*
T37	52.8*	46.6	95.9*	228.8
Control	63.0	53.0	122.1	241.9

* Significantly lower than control

TABLE V

Summary of the effect of seed treatment, from the data in Table II

Treatment	Emergence (%)	Converted emergence	Leaf length	Root length
Hulled oats				
Form. 1:320	90.0	74.2	202.3	433.8
Ceresan ½ oz.	91.7	78.6	212.9	437.2
Check	90.0	75.0	207.2	454.3
Hulless oats				
Form. 1:320	24.5*	28.5*	36.5*	105.0*
Ceresan ½ oz.	57.3	49.5	90.1	186.7
Check	62.3	52.6	116.3	219.0

* Significantly lower than check

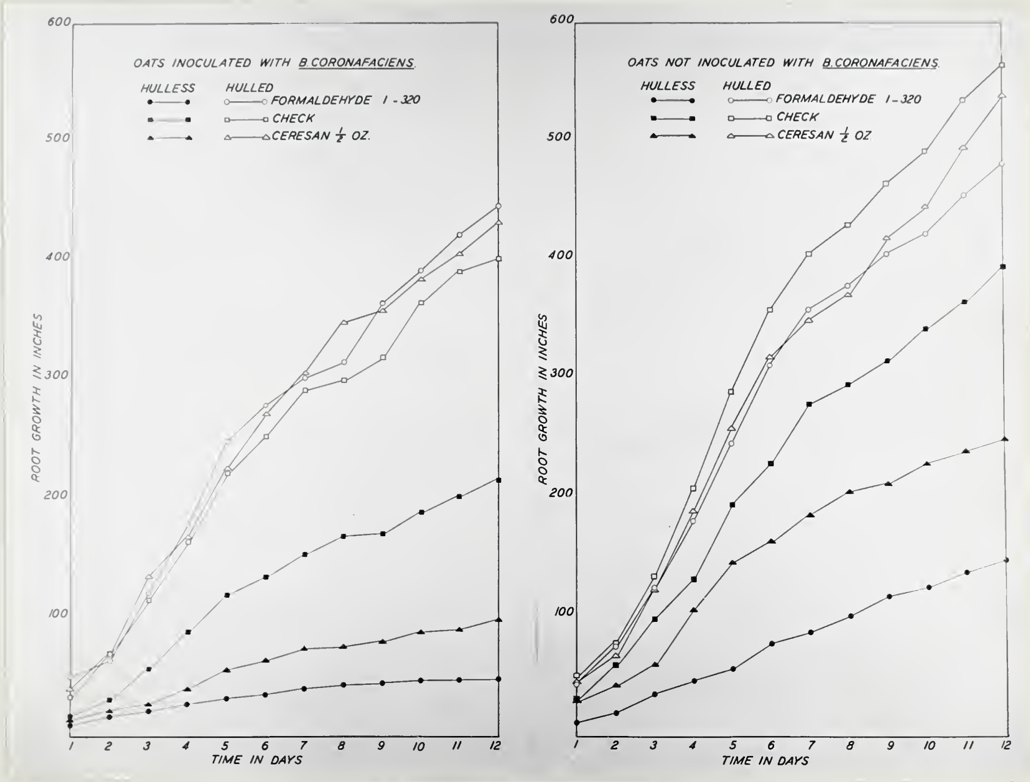

Figure 5

Effect of seed treatment on the daily root growth*
of Victory oats inoculated with B. coronafaciens

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*Total length per container.

which reduced the emergence, leaf length, and root length to a marked degree. Ceresan caused damage especially to hulless seed. This may be due to insufficient drying of the seed after inoculation and before treatment, which was found by Grimble (23) to increase the susceptibility of wheat to Ceresan injury. This is clearly illustrated in the graphs of the daily root growths shown in Figure 5.

In this experiment neither Ceresan nor formaldehyde had any beneficial effects on seed inoculated with oat blight isolates. The effectiveness of culture T33b in reducing emergence and root growth of hulled oats is illustrated in Figure 6a and the effect of treatment with Ceresan and formaldehyde on hulled, non-inoculated seed is shown in Figure 6b.

The experimental set-up was factorial in design and was analysed as such. It will be noted that the three errors are of the same magnitude, indicating that in this particular experiment the soil heterogeneity and other environmental conditions could be controlled enough so that the required information could have been obtained just as well from a simple randomized block, providing that similar precautions were taken in the preparation of the test.

The minimum significant differences were calculated for the treatments in general.

The emergence data are expressed in terms of percentage, but the extremely low value for formaldehyde treatment on hulless seed makes the validity of the analysis questionable (27). Hayes and Immer point out that if the

Inoculated with T33b Untreated | No inoculation Untreated | Inoculated with T37 Untreated

Figure 6a

The effect of inoculation with two isolates of B. coronafaciens in lowering the emergence and root growth of seedlings from hulled oats. Photographed 12 days after seeding.

Formaldehyde treated Not treated Ceresan treated

Figure 6b

The effect of seed treatment on the root growth of seedlings from uninoculated hulled oats. Photographed 12 days after seeding.

percentage range is between about 25 and 75, no modifications are necessary. Inasmuch as the range for these emergence data is between 10 and 100, the figures were converted to $\sin^2 \theta$, using the tables by Bliss (27, p.421-3). This conversion reduces the difference by increasing the size of the smaller values and reducing the size of the larger (23). Thus the significance of the Hull x Inoculation and Treatment x Inoculation interactions was removed so that the emergence data present a similar picture to those for leaf and root growth.

Effect of Seed Treatment on Victory Oats Inoculated with *B. coronafaciens*

A similar experiment to the foregoing was made using clean Victory oats obtained from the Cereal Division, and culture of *B. coronafaciens* T47. The moisture content of the soil for this test was lowered considerably. After adjusting to a 22% moisture content, the soil was placed in the glass-fronted containers and seeded in the usual manner with inoculated and non-inoculated oats treated with Ceresan, Spergon, and formaldehyde. Ceresan was applied at the rate of 1/2 oz. per bushel and Spergon at 2 oz. per bushel. The formaldehyde concentration used was 1:320. The surface of the soil was watered with 250 cc. of tap water and the cans were slanted at 45 degrees in the special wooden frames.

Results

Results for germination, emergence, moldy seeds, infected leaves, root length and leaf growth are summarized in Table VI. The mean squares are presented in Table VII, and the daily root growth is given in Appendix II.

TABLE VI

Effect of seed treatment on Victory oats inoculated with B. coronafaciens

Treatment	Germination (%)	Emergence (%)	Difference	Moldy seeds (%)	Infected leaves (%)	Length (ins.) After 11 days Root	Length (ins.) After 11 days Leaf
Inoculated							
Check	86	81	5	3	9	688	156
Form.	88	84	4	4	1	665	154
Ceresan	92	88	4	1	2	734	156
Spergon	84	80	4	0	7	658	147
Non-inoculated							
Check	98	95	3	0	0	810	190
Form.	95	87	8	5	0	679	167
Ceresan	95	94	1	0	0	851	178
Spergon	98	96	2	1	0	765	176
Min. sig. diff.	4.5	6.4				42.9	11.7

The root growth was found to be much more satisfactory on the whole than in the preceding experiment, and the bottom of the cans was reached in 12 days after seeding. The mold growth, usually troublesome, especially on the

TABLE VII

Analysis of variance for seed treatment on Victory oats inoculated with *B. coronafaciens*

Variance due to	D.F.	Mean square Germination	Emergence	Root growth	Leaf growth
Replicates	3	23	87	12263**	843**
Inoculations	1	612**	722**	64614**	4802**
Treatments	3	10	35	21404**	268
Inoc. x Treat.	3	46	83	5212	2027**
Error	21	20	41	1844	136
Total	31				

* Significant to the 5% point
** Significant to the 1% point

formaldehyde treated seeds was negligible, indicating that this factor was more important in very moist soil.

Inoculation with *B. coronafaciens* was effective in lowering the germination, emergence, root and leaf growth. Of the chemical seed treatments, only the Ceresan treatment produced a beneficial effect on the inoculated seed.(Fig.7a and 7b).

Effect of Seed Treatment on Infection of Victory oats by seed-borne inoculum of *B. coronafaciens*, in sterilized and unsterilized soil

Earlier experiments in the controlled temperature tanks gave variable results in so far as the effect of

Figure 7a

The effect of ceresan treatment on emergence and root growth of oat seed inoculated and uninoculated with <u>B. coronafaciens</u>

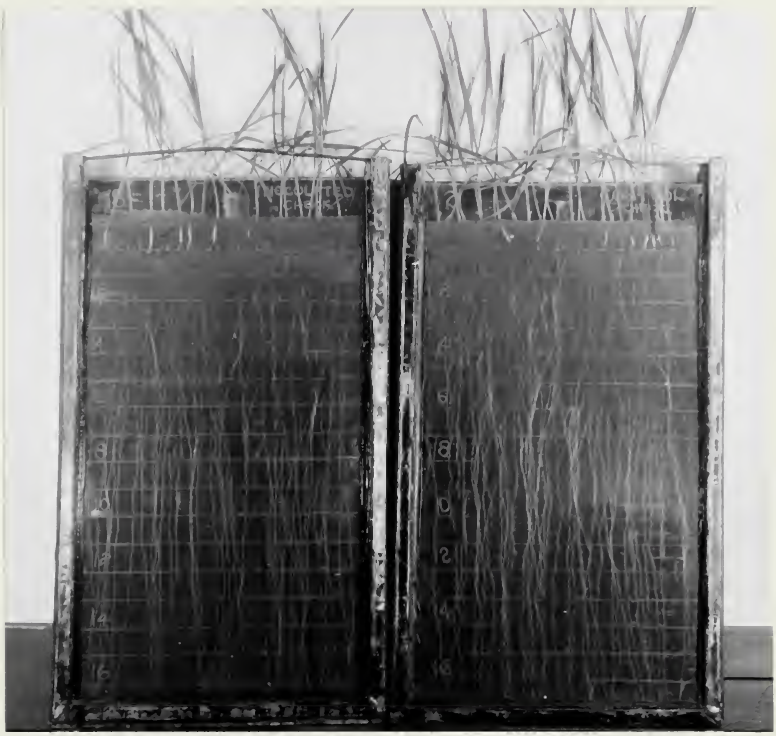


Figure 7b

The effect of inoculation of oat seed with
<u>B. coronafaciens</u> in reducing emergence
and root growth

sterilized soil was concerned. Since the temperatures of the greenhouse were well within the range of the pathogen, a greenhouse pot experiment was made to determine whether soil saprophytes have any effect upon the amount of infection by the seed-borne pathogen. Culture T47 was again used on Victory oats.

Method

Six-inch pots were filled with a 3:1 mixture of screened soil and sand. Half of the number were covered with paper and placed in the steam sterilizer for five hours at 15 pounds pressure. Both series were sown, 25 seeds per pot, with inoculated and uninoculated oats, treated with formaldehyde, Ceresan, and Spergon. The pots were then watered with sterile water and the covers were replaced. These were removed when the plants started to emerge, and subsequent waterings were made with unsterilized water.

Emergence and leaf infection notes were taken 21 days after seeding.

Results

The results are summarized in Table VIII, and the analysis of variance is given in Table IX.

Ceresan and Spergon gave markedly higher numbers of normal seedlings, but the leaf infection ratings were somewhat higher than in the checks. The seedlings from non-inoculated

seeds produced no leaf lesions and the per cent emergence was not lowered. The inoculated seed again produced poorer results in spite of seed treatment with Ceresan, Spergon, and formaldehyde. These effects were greater in unsterilized soil than in sterilized soil, which may indicate some synergystic effects by soil microorganisms to B. coronafaciens.

TABLE VIII

Effect of seed treatment on infection of Victory oats by seed-borne inoculum of B. coronafaciens in sterilized and unsterilized soil

	Sterilized soil			Unsterilized soil		
Treatment	Emergence (%)	Leaf infection (%)	Normal seedlings (%)	Emergence (%)	Leaf infection (%)	Normal seedlings (%)
Inoculated						
Check	80	3.3	77	72	10.0	62
Form. 1:320	80	1.6	78	73	10.0	63
Ceresan $\frac{1}{2}$oz.	89	6.6	82	87	8.2	79
Spergon 2oz.	90	5.0	85	85	6.6	78
Non-inoculated						
Check	100	0	100	100	0	100
Form. 1:320	100	0	100	98	0	98
Ceresan $\frac{1}{2}$oz.	100	0	100	100	0	100
Spergon 2oz.	100	0	100	100	0	100
Min. sig diff.	7		8	11		12

TABLE IX

Analysis of variance for seed treatment on infection of Victory oats by seed-borne inoculum of B. coronafaciens in sterilized and unsterilized soil

Variance due to	D.F.	Sterilized soil: Emergence (%)	Sterilized soil: Normal emergence (%)	Unsterilized soil: Emergence (%)	Unsterilized soil: Normal emergence (%)
Replicates	3	8	20	62	59
Inoculation	1	1891**	3042**	3321**	6962**
Treatment	3	58	27	136	171
Treat. x Inoc.	3	58	27	109	140
Error	21	24	35	64	72
Total	31				

Discussion

The organism in these studies had three distinct effects upon oat seeds and seedlings: (1) It prevented germination of inoculated seeds to a significant degree. Such seed apparently was predisposed to attack by molds to a greater extent than clean seed. This was clearly observable through the glass fronts of the containers used, as illustrated in Figure 6; (2) It caused a seedling blight which often killed young plants up to five or six inches in height, as shown in Figure 3; and (3) It caused isolated lesions which, under greenhouse conditions, did not affect the newer growth. In the field, however, under suitable weather conditions, lesions such as these are probably the most important source

of inoculum for secondary infections.

Seed treatment with Ceresan, Spergon, and formaldehyde was not completely effective in the control of the pathogen in any of the experiments. Though Ceresan gave consistently higher results in emergence, leaf and root growth on inoculated seed, the level of the non-inoculated check was not reached, indicating that the control effects were incomplete. Formaldehyde and Spergon likewise failed to give complete control.

EFFECT OF SOIL ENVIRONMENTAL FACTORS ON PRIMARY INFECTION

Since the causal organism of Halo Blight is seed-borne, the soil environment at the time of germination may be a factor in influencing the extent of primary infection. Controlled temperature tanks were available and were used to determine whether soil temperature affects the severity of the disease in the seedling stage of the host plant. The effect of the application of lime to the soil, on primary infection, was also investigated under different soil environmental conditions.

Effect of Soil Temperature on the Severity of Primary Infection of Victory Oats by *B. coronafaciens* in Sterilized and Unsterilized Soil

whether soil temperature is a factor in the development of infection of oat seedlings from seeds inoculated with B. coronafaciens. Sterilized and unsterilized soil was used.

Method

Constant temperature tanks were adjusted to 18°C, 22°C, 26°C, and 30°C. An equal quantity, namely 3800 grams, of a 3:1 mixture of Edmonton soil and sand adjusted to 16.6% moisture was added to each of 32 earthenware crocks. Half of these were sterilized at 15 pounds pressure for eight hours, after watering and covering with paper to prevent contamination.

Victory oats, obtained from the Cereal Division, were inoculated as previously described with Culture 14, isolated in 1941 from Erban oats obtained from Morningside and found to be very virulent. This seed, together with uninoculated seed used for controls, was seeded 10 to a pot.

Daily temperature recordings were made at 8:00 a.m. and 5:30 p.m. The fluctuations of the soil temperatures were considered to be negligible.

Results

The results are tabulated in Table X.

The controls showed no infection of the leaves in either sterilized or unsterilized soil. The infection ratings were generally higher and the emergences were generally lower

TABLE X

Per cent emergence and leaf infection of oat seedlings from inoculated seed in sterilized and unsterilized soil

	Inoculated			Non-inoculated	
Temperature (°C)	Emergence	Leaf infection	Normal seedlings	Emergence	Leaf infection
Sterilized soil					
18	60	50	10	100	0
22	80	55	45	80	0
26	80	44	41	95	0
30	75	52	43	80	0
Unsterilized soil					
18	85	32	53	100	0
22	80	44	41	95	0
26	95	47	48	100	0
30	80	12	68	95	0

in the former than in the latter. This may be due to the fact that antagonistic organisms in the soil which may tend to reduce infection have been destroyed by the soil treatment.

The range for infection appears to be very wide, temperatures from 18°C to 30°C being suitable for seedling infection.

Effect of lime applications to the soil on the severity of primary infections of Victory oats by B. coronafaciens to

It has been noted by Derick (10) that "The addition of lime to the soil appears to favor the development

of the organism .....", but no references are cited in support of this statement. An experiment was made to determine whether such treatment was favorable to seedling infection.

Method

The soil-temperature tanks were adjusted to 15°C, 18°C, 21°C, and 26°C. Finely powdered lime was added at the rate of 1:400 to 3:1 Edmonton soil and sand, and well mixed. The same mixture without lime was used for the controls. Both lots were brought up to about 26% moisture. Records of the total weight of the crock and soil were kept and the moisture content was kept approximately constant by the addition of water every two days.

Inoculated seed and the checks were prepared in the usual way. Culture No. 47 was used and 25 seeds were planted in each pot. The greenhouse temperature was adjusted to 65°C.

Results

The results are noted in Tables XI and XII.

The results of these experiments are variable and inconclusive. Lime application seems to reduce infection somewhat. Infection appears to be more severe in unsterilized soil. This is opposite to the effect obtained in the first experiment and may be due to an interaction between the lime and the microflora or the use of soils from different

sources may account in part for this inconsistency.

TABLE XI

Per cent emergence and leaf infection of oat seedlings in limed and unlimed soil

	Inoculated			Non-inoculated	
Temperature (°C)	Emergence	Leaf infection	Normal emergence	Emergence	Leaf infection
Lime					
15	84	14	70	100	0
18	84	10	74	96	0
21	72	2	70	98	0
26	72	2	70	94	0
No lime					
15	80	14	66	100	0
18	84	16	68	98	0
21	78	14	64	94	0
26	88	22	66	96	0

TABLE XII

Effect of lime on emergence and infection of Victory oat seedlings inoculated with B. coronafaciens and growing in sterilized and unsterilized soil

Temperature (°C)	Sterilized soil Emergence (%)	Sterilized soil Leaf infection (%)	Sterilized soil Normal seedlings (%)	Unsterilized soil Emergence (%)	Unsterilized soil Leaf infection (%)	Unsterilized soil Normal seedlings (%)
Lime						
14	84	0	84	74	4	70
18	74	6	68	74	6	68
22	72	8	64	72	8	64
28	78	0	78	72	12	60
No lime						
14	78	6	72	78	8	70
18	82	10	72	80	4	76
22	74	6	68	80	12	68
28	70	2	68	82	12	70

Discussion

In both culture media and in soil, the temperature range of B. coronafaciens was found to be relatively wide, being from 10°C to 35°C, and from 14°C to 30°C respectively. The addition of lime to the soil appeared to have a slight effect in lowering seedling infection instead of raising it as claimed by Derick (11).

The indications are that the damage from seed-borne B. coronafaciens is generally greater in unsteri-

lized soil than in sterilized soil, possibly owing to synergystic effects of soil organisms. This aspect of microorganic relationships is further considered in the next section on antagonistic relations.

ANTAGONISTIC RELATIONS OF SOME MICROORGANISMS TO *B. CORONAFACIENS*

Introduction

Microorganisms growing together in artificial culture media may exert a synergistic association effect, or an antagonistic one. From the standpoint of plant diseases, these relations, providing they hold under natural conditions, may be of some practical importance, inasmuch as the presence of one or more other organisms may render a plant pathogen more or less virulent. For example, White and McCullough (56) showed that the presence of an associated bacterium with *Bacterium hederae* increased the damage to ivy, while the presence of other associated bacteria decreased the damage.

Considerable work on the isolation of antagonistic substances from microorganisms has been done in the medical field. Dubos (14) working with the antibacterial products of *Bacillus brevis*, and Fleming (18) with those of *Penicillium notatum* found fractions more toxic to certain pathogenic organisms than the most powerful bactericides known.

In the field of Plant Pathology the early work on this line was done by Potter (40) who, using a staled turnip broth culture of _Pseudomonas destruens_, the causal organism of white rot of turnip, was able to demonstrate that toxic metabolic products therefrom were capable of killing this pathogen and preventing rot, when sprayed on the turnip. Lee (33) noted that _Pseudomonas citri_ disappeared in unsterilized soil within six days, while it increased rapidly in sterilized soil. He attributed this to the deleterious action of the soil microorganisms. This was further substantiated by the work of Fulton (19). Porter (39) by using antagonistic bacteria was able to demonstrate the protection of wheat seedlings from _Helminthosporium_ sp. and of flax from _Fusarium_ sp.

That the soil microorganisms play an important part in the control of cereal pathogens causing foot and root rots was shown by Henry (28), who demonstrated that the natural microflora of the soil was capable of inhibiting the growth of such organisms as _Helminthosporium sativum_ and _Fusarium graminearum_. Sanford and Broadfoot (43) and Broadfoot (6) have also shown that various bacteria are capable of inhibiting certain plant cereal pathogens, especially _Ophiobolus graminis_.

King _et al_ (32) attributed the beneficial effects of the addition of organic manures to the soil for the control of root rot of cotton under continuous cultivation and irrigation to the antagonistic action of other soil microorganisms on the causal fungus, _Phymatotrichum omnivorum_.

The work of Weindling (52), Weindling and Emerson

(54) and Allen and Haenseler (2) on the preparation of toxic substances from fungi, indicates that these products are important factors in producing the antibiotic effects. Broadfoot (6) points out that organisms antagonistic to _Ophiobolus graminis_ in culture were not always antagonistic in the soil, and vice versa. Thus the growth reaction on artificial media cannot always be considered to be a reliable index of antagonism in the soil. Similarly Christensen and Davies (8) point out that while bacteria and fungi may have a distinctly antibiotic effect on certain fungi in culture media, in soil they may not, because the toxic substances may be adsorbed on the soil particles and hence become too dilute to be effective. Moreover, other microorganisms may neutralize or destroy this toxic substance. For example, _Botrytis cinerea_ growing on a bacterium staled medium was shown to destroy or inactivate the toxic substance produced by _Bacillus mesentericus_, while _Ustilago zeae_ had no such effect.

Cheudiakov (20) found _Achromobacter_ and _Pseudomonas_ spp. capable of lysing various soil fungi. He was able to protect wheat seedlings from attack by _Fusarium_ sp. by bacterizing them with lytic bacteria. Bamberg (4) and Johnson (31) suggest that the antibiotic effects of certain bacterial isolates on _U. zeae_ is due to the enzymes produced by the former.

Acid production, though depressing to certain organisms in culture media is not considered to be a large factor in antibiosis in the soil, inasmuch as the strong buffer

action of most soils nullifies any effect it might have (42).

This "antibiosis" or antagonistic relation between microorganisms had been generally attributed to the competition for food until De Bary in 1879 pointed out that this relation was of a much more complex nature. The production of toxins, lytic principles, enzymes, acids, and competition for nutrients have been claimed by various workers to account for this effect (50). It apparently is conditioned by many factors such as temperature, pH, oxygen relations, etc.

Waksman (51) has summarized the modes of interference of the normal processes by antibiotic substances as follows:

(1) Oxidizing a metabolic substance which has to be reduced in the process of bacterial nutrition;

(2) Rendering nutrients unavailable by combination;

(3) Competing for an enzyme required for bacterial metabolism;

(4) By affecting surface tension of bacteria;

(5) By influencing its respiratory mechanism;

(6) By interfering with bacterial cell division.

Broom (5) suggests that inhibition is caused by a deficiency of available carbon due to the original growth and thus the bacteria with greater fermentive powers inhibit those with less. Thjoita (49) claimed that inhibition was due to exhaustion of accessory food factors and that the addition of yeast extract would revivify old cultures. The addition of vitamins was claimed by Broadhurst to revivify old cultures, likewise, but Werkman (55) showed that the determining factor

was the nitrogen added in the vitamin, and that the same stimulation was present in the absence of the vitamin.

The relation may be "direct" or true antagonism, characteristic of the action of living cells as in the case of Bamberg's bacterial isolates which were antagonistic to the smut organism only while in the living state (4) or it may be "indirect" wherein the effects are due to the metabolic products injurious to other organisms, as in the case of Weindling's filtrates from *Trichoderma* and *Gliocladium* which were antagonistic to *Rhizoctonia*. Such products may be: (1) bacteriostatic, such as actinomycin from *Actinomyces antibioticus*; (2) bactericidal but not bacteriolytic, such as penicillin from *P. notatum*; pyocyanin from *Pseudomonas aeruginosa*; and glyotoxin from *Gliocladium*; (3) bacteriolytic such as gramicidin from *Bacillus brevis*.

The Application of Antagonism in Seed Treatment

The effects of antagonists and their metabolic products to plant pathogens in artificial culture media and in the soil has been widely studied, but little has been done on the utilization of their effects on seed-borne pathogens.

Previous work at this laboratory by Campbell (7) has shown that the spores of *Colletotrichum linicolum* are completely lysed by a short exposure to a soil bacterium.

He points out "that in many diseases caused by seed borne fungi, the surface-borne inoculum might be rendered inocuous by such action soon after the seed was sown, in this way preventing much initial infection."

Novogrudski *et al* (37) showed that the bacterization of diseased flax seed with soil bacteria capable of lysing *Fusarium* and *Colletotrichum* sp. significantly decreased the number of diseased seedlings growing from such seed. They concluded that bacterization gave some promise as an effective control treatment for seed-borne pathogens.

Henry and Campbell (30) noted that some seed-borne pathogens, such as the flax pathogen, *Polyspora lini*, are inactivated to a marked degree by the natural microflora of the soil, whereas others, such as certain smut fungi which attack cereals, are not.

The addition of antagonists to normal soil for the control of specific pathogens has usually given poorer results than expected, owing to the various soil effects such as the relations of other soil microorganisms, buffering capacity of the soil, adsorption of the toxic products by the soil particles. The direct application of antagonists or their products to seed might, however, be effective against certain seed-borne pathogens, as well as in protecting the seed from soil organisms.

Preliminary Tests with Two Antagonists on Selected Bacterial Phytopathogens

Two organisms, a yellow bacillus and *Bacillus vulgatus*, found by Ark and Hunt (3) to be strongly antagonistic to a large number of phytopathogenic bacteria and fungi were obtained from the authors and tested on *B. coronafaciens* and on several other bacterial plant pathogens available in the laboratory collection. Suspensions of the pathogen to be tested were added to potato dextrose agar at 42°C, poured into plates and cooled. Streaks were then made with the test organisms. The inhibitory power was measured by the extent of inhibition as indicated by the clear zone surrounding the streak. The general results are compared with Ark's in Table XIII and illustrated in Figure 8.

It is apparent that these two antagonists differ widely in their action against other bacteria. For example, though *B. vulgatus* completely inhibits *B. atrofaciens*, the yellow bacillus has no such effect. Yet both antagonists almost completely inhibit *A. sepedonicum*. In general the yellow organism is far inferior in action to *B. vulgatus*.

TABLE XIII

Antagonism in artificial culture media of *B. vulgatus* and a yellow bacillus on some bacterial plant pathogens

	Inhibitive effects of antagonists			
	Yellow bacillus (A9)		B. vulgatus (A10)	
Plant pathogen	I	II*	III	IV*
Bacterium atrofaciens	0	-	++++	-
Bacterium campestre	+	+	+++	+
Bacterium coronafaciens	++	-	+++	-
Bacterium phaseoli	+	-	++	-
Bacterium tumefaciens	0	+	++	+
Bacterium striafaciens	++++	-	++++	-
Bacterium woodsii	0	-	+	-
Bacillus carotovorus	0	+	+	+
Aplanobacter sepedonicum	+++	+	+++	+

+ antagonistic
0 not antagonistic
- not tested
* results reported by Ark.

NOTE: Cultures A9 and A10 kindly provided by Dr. P. A. Ark, University of California

Antagonism of Other Bacterial Plant Pathogens to *B. coronafaciens*

An experiment was made to determine whether any of the other bacterial plant pathogens that were available were capable of an inhibiting action on the Halo Blight organism.

Plates were poured from a potato dextrose agar suspension of *B. coronafaciens* and after cooling were streaked with the nine pathogens used in the previous study.

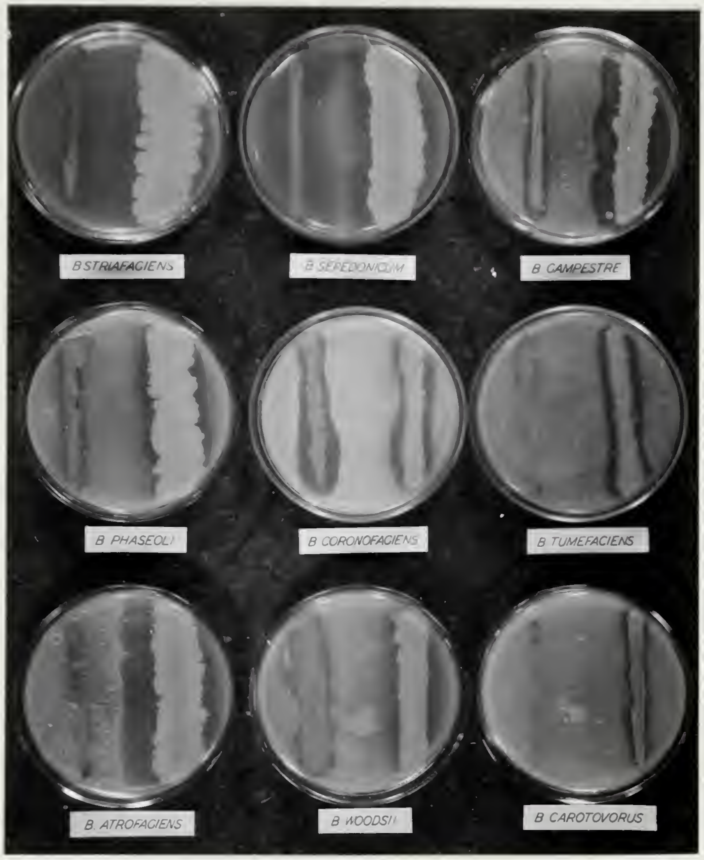


Figure 8

The relative inhibitive action of two bacterial isolates supplied by Dr. P.A. Ark, University of California, on B. coronafaciens (centre) and on several other plant pathogenic bacteria.

Streak on left in each plate = A_9, a yellow baccilus
Streak on right in each plate = A_{10}, B. vulgatus

Little indication of inhibitory action as indicated by the formation of sterile zones around the streaks was shown by any of these organisms. It was concluded that the production of substances inhibitory to B. coronafaciens was not a property of any of the bacterial pathogens tested.

Antagonism of Some Spore Formers to B. coronafaciens

The property of producing bactericidal substances is considered to be a characteristic of many groups of soil spore-formers. Cultures of Bacillus cereus, Bacillus megatherium, Bacillus, mycoides, Bacillus subtilis, and Bacillus vulgatus were available. Together with an isolate of B. subtilis from the Department of Dairying, these were compared with the two organisms obtained from Ark, for their antagonistic action against B. coronafaciens.

Little or no antagonism was shown by any of these isolates with the exception of Ark's two type organisms. The laboratory culture of B. vulgatus did not show the antagonistic action exhibited by the one supplied by Ark from California. This may indicate some difference in physiologic strains of the same organism in ability to produce antagonistic substances.

Antagonism of Soil Actinomycetes to *B. coronafaciens*

The production of powerful inhibitors by certain soil actinomycetes to plant pathogens has been reported. A number of actinomycetes were isolated from Alberta soils representative of the black, brown, and grey wooded soils. These were tested for antagonistic effects by inoculating plates of potato dextrose agar suspensions of *B. coronafaciens*.

While many of the actinomycetes produced pigments that rapidly diffused into the surrounding media, little or no inhibitory effect was apparent. Some showed a very slow lytic action on colonies of the oat pathogen surrounding the inoculum, but in general these organisms were considered to be of little value.

Antagonistic reactions of Isolates from Soil and Plant Tissue to *B. coronafaciens*

The most promising antagonistic organisms found were certain bacteria isolated from dilutions of soil and macerated plant tissue. These were tested as before on potato dextrose agar suspensions of the Halo Blight organism. The best of these, as evaluated by the extent of the zone of inhibition, were retained and compared with Ark's organisms for their inhibitory action. The sources and inhibitory ratings of the

selected isolates are shown in Table XIV.

TABLE XIV

Sources and inhibitory ratings of several bacterial antagonists, isolated from soil and plant tissues to <u>B. coronafaciens</u>

Antagonist	Source	Color of colony	Inhibitory rating
A2	wheat glume	buff with pink pigment	+++
A6	soil	cream	+
A8	oat glume	lemon yellow	++++
A9*	soil	cream	++
A10*	soil	orange yellow	++
A13	soil	pink	++
A14	oat glume	yellow	+++
A25	oat leaf	white	++++
A32	oat leaf	yellow	+++
A41	oat glume	yellow	++
A42	oat glume	yellow	++++

* A9 = <u>B. vulgatus</u> and A10 = yellow bacillus, isolates provided by Dr. P. A. Ark.

The production of the sterile zones was apparent in two to four days of incubation at room temperature. At least six isolates were obtained which were considered superior antagonists to the two organisms, namely A9 and A10 obtained from Dr.P.A.Ark and used for test purposes (see Figure 9).

To determine whether the clear areas around the antagonists contained inhibitory substances, circles of agar were cut out of these zones of inhibition with a small sterilized cork borer, and planted on potato dextrose agar

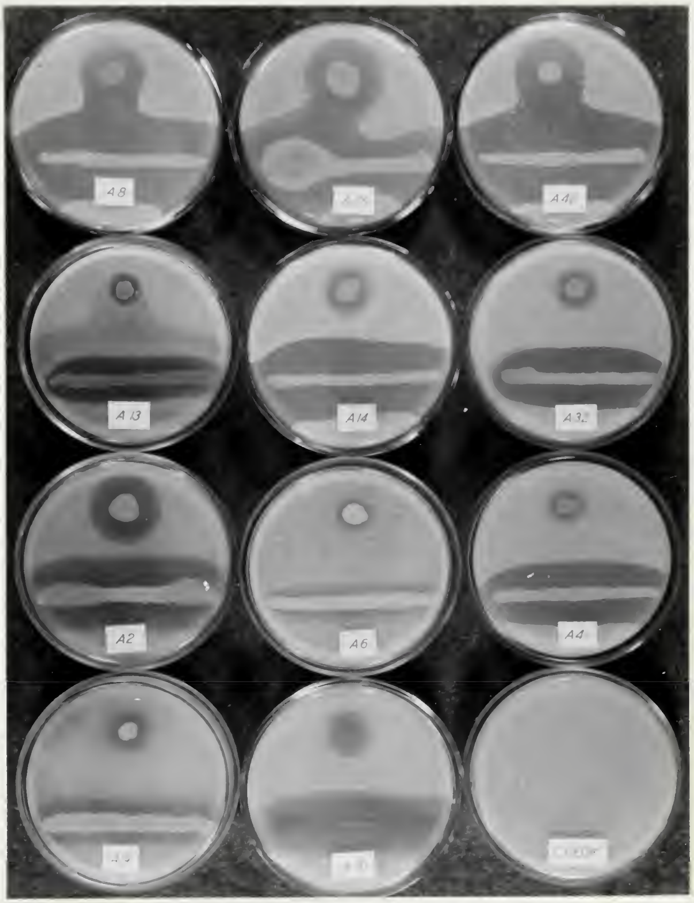


Figure 9

Inhibitory effects of bacterial isolates from soil and plant tissues on B. coronafaciens on potato dextrose agar. A_9 and A_{10}, the test organisms from Dr. Ark are included for comparative purposes. The check was spot and streak inoculated with sterile water.

suspensions of B. coronafaciens, and incubated at room temperature. Circles from the sterile zones of A42 were the only ones which gave even a slight indication of a residual inhibitory action. A marked contrast was shown by the distinct and clear-cut circular zones produced by circles contaminated with the antagonist. Thus, it is fairly certain that the living cultures will be more effective in antibiosis than the metabolic products, in so far as these isolates are concerned.

Four general reactions of other bacteria on plates of B. coronafaciens were noted: (1) the organism itself was inhibited as shown by the sparse growth along the inoculation streak; (2) the organism was compatible with the pathogen, and was in some cases stimulated as shown by the good growth along the streak; (3) the organism displayed a bacteriostatic action, whereby the growth of the pathogen was merely checked, as illustrated by isolate A6 in Figure 9; (4) the organism inhibited the pathogen, in which case the line of demarcation between the sterile zone and the region of growth of B. coronafaciens was well marked. This is also shown in Figure 9 by the isolates in the top row, A8, A25, and A42.

Effect of Bacterial Antagonists on Oats Inoculated with B. coronafaciens

In the culture media experiments a number of

isolates of bacteria were found to be far more effective antagonists of B. coronafaciens than the ones obtained from California for comparative purposes. Two of the best, A8 and A42 were compared with these California isolates, designated A9 (B. vulgatus) and A10 (yellow organism), for their effect upon the Halo Blight organism applied to the oat seed.

Method

A sample of Victory oats was inoculated with culture T47 of B. coronafaciens in the manner previously described. The inoculated seed was dried at room temperature for four days. Inoculated and non-inoculated seed was then soaked in separate water suspensions of the antagonists A8, A9, A10, and A42, and some in sterile water to serve as checks. The seed was finally dried at room temperature for two days.

The glass-fronted containers were then filled with a 4:1 mixture of Edmonton soil and sand, adjusted to a 21% moisture content. The seed was sown, 25 to a can, one inch deep, embryo pointing downwards and against the glass. The containers were then watered with 250 cc. of water and placed in wooden frames at an angle of 45 degrees. Daily recordings of the root growth were made as previously described.

Results

The results are given in Table XV and the summary of the analysis of variance shown in Table XVI. The daily root

growth is tabulated in Appendix III.

TABLE XV

Effect of bacterial antagonists on oats inoculated with B. coronafaciens

Treatment	Germination (%)	Emergence (%)	Difference	Moldy seeds (%)	Infected leaves (%)	Length (ins.)	
						Roots	Leaves
Inoculated							
Check	83	73	10	13	5	564	167
A8	83	79	4	7	7	531	160
A9	83	75	8	12	4	649	151
A10	81	75	6	9	1	582	158
A42	93	91	2	3	3	671	196
Non-inoculated							
Check	99	96	3	0	0	619	191
A8	99	93	6	0	0	705	208
A9	100	100	0	3	1	719	198
A10	100	99	1	1	0	663	207
A42	100	100	0	0	0	757	220
Min. sig. diff.	5	8				83	20

TABLE XVI

Analysis of variance for effect of bacterial antagonists on oats inoculated with *B. coronafaciens*

Variance due to	D.F.	Mean square: Germination	Emergence	Leaf growth	Root growth
Replicates	2	19	1	1213	31683**
Inoculations	1	1673**	2765**	10932**	65240**
Treatments	4	42	107	1011*	13102
Inoc. x Treat.	4	113**	71	287	5787
Error	18	20	50	308	5216
Total	29				

* Significant to the 5% point
** Significant to the 1% point

Inoculation with *B. coronafaciens* was effective in producing the symptoms of the disease and a general reduction in emergence and growth of the seedlings from non-bacterized seed (Figure 10).

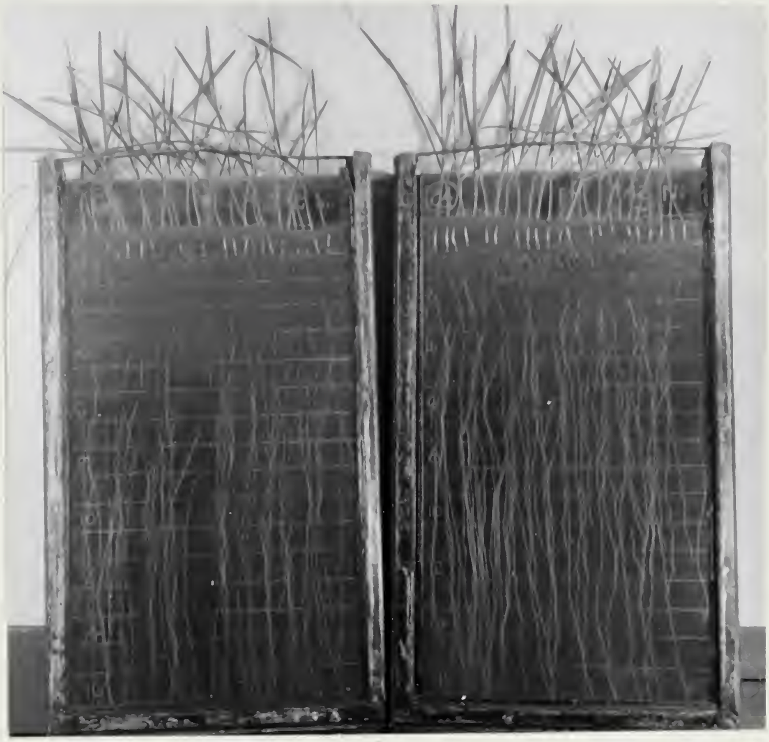
The antagonists varied in their effects upon the contaminated seed, but A42, the yellow organism isolated from an oat glume, was the only one which induced a marked increase in germination, emergence, root and leaf length. The relatively little difference in damage between contaminated and non-contaminated seed when bacterized with A42 is illustrated in Figure 11. The marked improvement in emergence and root growth as a result of bacterization is apparent when Figure 11A is compared with Figure 10A.

Not bacterized

A. Inoculated seed B. Non-inoculated seed

Figure 10

The damaging effect of inoculating the seed of Victory oats with B. coronafaciens



Bacterized with A42

A. Inoculated seed B. Non-inoculated seed

Figure 11

The effect of bacterization of the seed of Victory oats with isolate A42 in reducing damage by B. coronafaciens

The high germination of contaminated seed treated with A42, and the high survival of the seedlings therefrom indicate a high protective value by A42. This is further shown by the relatively small percentage of seedlings attacked by molds. The antagonistic action, however, was not complete, inasmuch as the typical symptoms of seedling infection caused by *B. coronafaciens* appeared on 3% of the plants.

None of the other antagonists showed any significant beneficial or detrimental effects, although there is some indication that A8 increased the damage by reducing the root growth somewhat.

In spite of the relatively large amount of inoculum added, the disease symptoms as expressed by leaf lesioning were not sufficiently severe to show clearly differential effects resulting from bacterization. The germination and, to a lesser extent, the emergence appear to give a good index of the damaging effect of contamination of the seed with *B. coronafaciens*.

The Effect of Fungal Isolates on Oats Inoculated with *B. coronafaciens*

Many of the common fungi are also known to have antagonistic effects on specific plant pathogens. It has been noted that the production of pigment is often associated with antagonistic properties. A number of pigment producers

were therefore isolated and tested on B. coronafaciens carried upon the oat seed. Trichoderma 1001, found to be antagonistic toward O. graminis and other fungi by Ludwig (34), was used for comparative purposes.

Isolates A12, A18, and A26 were obtained from soil dilution plates, Ac from manure, and Ao from a contaminated culture. All were Penicilliums with the exception of A26 which was a species of Aspergillus.

Method

The inoculated seed was prepared as for the former experiment with bacterial antagonists. Heavy spore suspensions of Trichoderma 1001, Penicilliums Ac, Ao, A12, A18, and Aspergillus A26, grown on 2% potato dextrose agar, were applied to separate lots of inoculated and non-inoculated seed. This was then air dried at room temperature for two days.

The seed was sown in six-inch pots containing a 3:1 mixture of Edmonton soil and sand mixture. One half of the pots of soil were sterilized and the other half left unsterilized. The sterilized series was prepared by covering the pots with paper and autoclaving for eight hours at 15 pounds pressure. After sowing the pots were watered with sterile water. The paper covers were retained on the sterilized pots and were removed when the seedlings began to emerge.

Data on emergence and leaf infection were taken 21 days after seeding.

Results

The results are summarized in Table XVII and the analysis of variance in Table XVIII. A highly significant decrease in emergence resulted from inoculation with B. coronafaciens. The non-inoculated series shows no disease symptoms as expected, while the inoculated series shows a varied reaction to the addition of the different fungi.

In sterilized soil all the fungal isolates tended to reduce leaf infection, but in unsterilized soil A18 and A26 tended to increase it, possibly owing to some synergystic effect. Penicilliums Ao and A12, however, gave promising indications of increasing emergence and decreasing leaf infection. Trichoderma 1001 did not appear to produce the antagonistic effects it does against fungus pathogens.

TABLE XVII

Effect of fungal isolates on oats inoculated with B. coronafaciens

Treatment		Emergence (%)	Leaf infection (%)	Normal seedlings (%)
Sterile soil				
Inoculated	Check	88	12	76
	Trichoderma 1001	89	3	87
	Penicillium Ac	91	8	83
	Penicillium Ao	100	1	99
	Penicillium A12	99	0	99
	Penicillium A18	89	5	84
	Aspergillus A26	83	7	76
Non-inoculated	Check	100	0	100
	Trichoderma 1001	95	0	95
	Penicillium Ac	96	0	96
	Penicillium Ao	95	0	95
	Penicillium A12	100	0	100
	Penicillium A18	100	0	100
	Aspergillus A26	97	0	97
Unsterilized soil				
Inoculated	Check	83	7	79
	Trichoderma 1001	89	3	87
	Penicillium Ac	85	3	83
	Penicillium Ao	99	0	99
	Penicillium A12	99	4	95
	Penicillium A18	93	9	84
	Aspergillus A26	91	9	81
Non-inoculated	Check	99	0	99
	Trichoderma 1001	97	0	97
	Penicillium Ac	100	0	100
	Penicillium Ao	93	0	93
	Penicillium A12	99	0	99
	Penicillium A18	95	0	95
	Aspergillus A26	96	0	96
Minimum significant difference				12.4

TABLE XVIII

Summary of analysis of variance for effect of fungal isolates on oats inoculated with B. coronafaciens

Variance due to	D.F.	Mean square: Emergence	Mean square: Normal seedlings
Soil	1	3	-
Replicates	2	3	24
Error (1)	2	3	14
Inoculation	1	732**	2432**
Inoc. x Soil	1	3	9
Error (2)	4	8	15
Treatment	6	80	175
Treat. x Soil	6	459	18
Treat. x Inoc.	6	332	262**
Treat. x Inoc. x Soil	6	383	10
Error (3)	48	443	28
Total	83		

Discussion

All the phytopathogenic organisms tested by Ark were not available. Of those that were, the results were substantially the same when treated with B. vulgatus but not with the yellow organism. The latter was reported to be antagonistic to both B. carotovorus and B. tumefaciens, but this could not be confirmed with our cultures of these organisms.

A marked difference is shown in the reaction of the bacterial plant pathogens to these two antagonists, ranging

from no inhibition to complete inhibition. B. vulgatus is generally far more efficient in this respect than the yellow organism. This is illustrated in Figure 8. A. sepedonicum and B. striafaciens are almost completely inhibited by both antagonists, but B. atrofaciens is completely inhibited only by B. vulgatus. This illustrates specificity of antibiotic reactions.

A number of isolations from plant tissue and soil dilutions were made that exhibited far greater antagonism toward B. coronafaciens on culture media than the antagonists obtained from Ark. Most of the effective isolates were chromogenic, though an especially good white organism was obtained. Selected plant pathogens, the B. subtilis and the soil actinomycetes used were not so successful in the production of antibiotic substances.

Evidence of protective action by bacterial and fungal antagonists to seed contaminated with B. coronafaciens was found. Bacterial isolate A42 and Penicillium A26 were considered to be especially effective in reducing pre-emergence blight and, to a lesser extent, seedling leaf infection. In this respect these particular isolates were far superior to the antagonistic organisms used for comparative purposes in these tests, namely B. vulgatus and a yellow organims used by Ark and Hunt (3), and a Trichoderma used by Ludwig (34).

It is suggested that the application of suspensions of specific bacterial and fungal microorganisms to contaminated seed gives some promise as a method for control of Halo Blight of oats.

EFFECT OF SEED TREATMENT ON THE OAT HOST PLANT AND ON OTHER CEREALS

Introduction

A great deal of work has been done on the effect of seed treatment on various pathogens without sufficient attention being given to the reactions of the seed of their host plants to the chemical used. Thus, in the previous studies for the control of B. coronafaciens, formaldehyde gave some measure of control but the damaging effects to the host plant of such treatment overshadowed the benefit due to this control.

These studies were undertaken chiefly to acquire information on the effect of seed fungicides such as formaldehyde and Ceresan on the host itself. Since the same fungicides as are used for oats are likely to be applied to other cereals on the farm, studies were also made with several of these for comparative purposes.

Considerable use of the glass-faced galvanized iron containers, as described in the previous experiments, was made. These allowed observations on the daily root growth and also on the protection offered by various chemicals as shown by the amount of mold attack and the time of pre-emergence blight of germinated seeds.

Finely screened 3:1 mixtures of Edmonton black soil

and sand were used in all the tests, unless otherwise indicated, at moisture levels as noted. The temperature of the greenhouse was thermostatically controlled at about 68° to 70°C.

Preliminary Experiment on the Effect of Seed Treatment with Ceresan and Formaldehyde on Oats, Barley, and Rye

A preliminary experiment was made using three cereal grains treated with formaldehyde 1:320 and Ceresan 1/2 oz. In the former treatment the seed was dipped in formaldehyde 1:320 solution for two minutes, drained, covered for four hours, and dried at room temperature. In the latter treatment, Ceresan at the rate of 1/2 oz. per bushel was shaken with 100-gram lots of the seed in small Erlenmeyer flasks, which were plugged with cotton bungs, and left overnight.

Victory oats, O.A.C. 21 barley, and Dakold winter rye were used. The seed was sown in the glass-fronted containers containing 8400 grams of a 3:1 soil and sand mixture. After sowing 1600 cc. of tap water were added, and the cans were placed in frames at an angle of 45 degrees.

Observations were started on the third day after seeding. The glass was marked off at one-inch intervals from the seed level, one inch below the soil surface. The number

of roots within each division was multiplied by the distance of the lower limit of the area thus marked off from the seed level. These results were then added to give an estimate of the total length of roots per container.

Results

The results are summarized in Table XIX, and the daily root growth is shown in Appendix IV.

TABLE XIX

Percent emergence and root growth in inches of cereal grains after seed treatment with Ceresan and formaldehyde

	Barley		Oats		Rye	
Treatment	Emergence	Root growth	Emergence	Root growth	Emergence	Root growth
Ceresan	96	153	98	380	66	179
Formaldehyde	90	162	80	198	16	35
Check	84	183	96	337	48	130
Minimum significant difference		-		51		37

Neither Ceresan nor formaldehyde had any significant beneficial or detrimental effects on the root growth of barley, although the emergence was increased by the former treatment. On oats, formaldehyde reduced root growth considerably. On rye, Ceresan had a marked beneficial effect, but formaldehyde had a very great detrimental effect on both emergence and

root growth.

Some difficulty was encountered by roots not growing close to the glass throughout their whole length, and thus observer error may account for the anomalies noted in the daily root growth. However, these were generally ironed out on averaging.

Effect of Seed Treatment with Different Concentrations of Ceresan on the Root Growth of Clean Oats and Wheat

The effect of different concentrations of Ceresan, varying from 1/2 oz. to 3 oz. per bushel, on the leaf growth and emergence of wheat was studied by Grimble (23). In general a definite increase in emergence and height at the recommended rate of 1/2 oz. was shown. A similar trend was obtained in the yield tests. Overdoses of Ceresan at rates higher than 1 oz. per bushel were found to be injurious when the moisture content of the seed was high, and when the treatment was carried out in tightly stoppered flasks.

This experiment was made chiefly to compare the effect on root growth of treatment with Ceresan at half the recommended rate with treatment at higher rates. If the low rate should be sufficient for protection, the cost of treatment would be reduced considerably.

Method

The glass-fronted galvanized iron cans previously mentioned were used. Each container was filled with 8400 grams of 3:1 air-dry mixture of Edmonton soil and sand, then watered with 1600 cc. of water. The pH of the soil was 6.1. Clean Victory oats and Red Bobs wheat, threshed as under ordinary farm conditions were the varieties tested. The percentages of wheat seed with damaged embryo covering was 17.5%.

Treatments at the following rates were made: 0, 1/4, 1/2, and 1 oz. per bushel. The grain was treated in 100-gram lots with weighed amounts of undiluted Ceresan. The seed was shaken with the Ceresan in glass bottles in a shaker machine for five minutes. It was then allowed to stand for 38 hours in wide-mouthed bottles covered with two layers of cotton cloth, to simulate ordinary methods of treatment.

The seed was sown, 25 seeds to a can, with the embryo pointing downwards and against the glass, at a depth of one inch.

The usual greenhouse space was not available for this experiment and a side bench was used instead. The location was such that half of the frames were close to an annex wall and the other half to a glass outside wall. It was observed that, in the early stages of germination, the difference between the 1/4 oz. Ceresan treatment and the check was more marked in the cans near the inside wall than in those next to the outside wall. The explanation probably lies in

the extremely cold weather at the time of this experiment. Though the temperature of the greenhouse section was thermostatically controlled at 68°F, the soil temperature of the cans close to the outside glass walls was about 8°F lower in the mornings than that in the others.

After the first root length readings were taken, the experiment was moved to another bench where the temperatures could be kept more constant throughout the block.

Results

The results are summarized in Table XX and the daily root growth is given in Appendix V.

The beneficial effects of the Ceresan 1/4 oz. on the root growth were especially noticeable on the second day after germination, but these differences were generally less apparent as the experiment progressed.

The treatment of clean Victory oats in the usual manner with Ceresan at different rates gave a significant increase of root growth only at the 1/4 oz. rate. Emergence and leaf length of oats were not appreciably affected by the treatments. With Red Bobs wheat, all the Ceresan rates gave marked increases in leaf length. The indicated improvements in emergence and root lengths, however, did not prove to be statistically significant under the conditions of this experiment.

TABLE XX

Effect of seed treatment with different concentrations of Ceresan on the root growth of clean oats and wheat

Treatment	Emergence (%)	Root length*	Leaf length*
Victory oats			
Ceresan 0 oz.	100	725	253
Ceresan $\frac{1}{4}$ oz.	100	794	250
Ceresan $\frac{1}{2}$ oz.	99	753	249
Ceresan 1 oz.	99	719	237
Minimum significant difference	-	49.0	-
Red Bobs wheat			
Ceresan 0 oz.	92	403	214
Ceresan $\frac{1}{4}$ oz.	98	439	230
Ceresan $\frac{1}{2}$ oz.	97	409	227
Ceresan 1 oz.	97	438	226
Minimum significant difference	-	-	6.6

* Average length per container, in inches, on the 11th day.

Effect of Seed Treatment on Injured and Uninjured Wheat and Oats

In the studies of the effects of seed treatment on grain, the condition of the seed should be taken into consideration. The amount of injury may vary with the variety and threshing methods. For example, grain threshed in a small rod-row machine may show a greater proportion of seed with broken or cracked embryo coverings than grain threshed with a

field machine. This injured seed may be expected to react to a greater extent than normal seed to the chemicals used in seed treatment.

Method with Wheat

Red Bobs wheat, threshed with a field machine was used. This was found to contain 25.6 per cent of damaged seed. To obtain the necessary number of damaged seeds for the test, artificial injury was resorted to, whereby the embryo covering was slit with a needle without damaging the embryo itself.

In the formaldehyde treatment the seeds were immersed in a 1:320 solution, drained and covered with cheesecloth for four hours and dried overnight. The dusts were added to the seed lots in Manila coin envelopes, shaken and also left overnight, each treatment separate from the others. Ceresan was applied at the rate of 1/2 oz. per bushel, and Nomersan and Spergon each at 2 oz. per bushel. The checks were similarly shaken and stored.

The glass-fronted cans were used, each filled with 8600 grams of finely sifted dry mixture of 3:1 Edmonton soil and sand. The seed was sown ten to a can, one inch deep, embryo pointing downwards and against the glass. The containers were watered by replicates with 2000 cc. of tap water, and placed in their frames.

Seed was considered to have germinated when the roots

had reached a length of at least 1/4 inch, and to have emerged when the coleoptile had broken through the surface of the soil.

Results

The results of the experiment are summarized in Table XXI. Daily notes on root growth and leaf growth, germination, and emergence are recorded in Appendix VI, VII, IX, and X, respectively. The results of the analysis of variance for the germination and emergence data are given in Table XXII, and for the leaf length and root length data in Table XXV.

TABLE XXI

Summary of experiment on seed treatment of injured and uninjured wheat

Treatment	Germination (%) 14th day	Emergence (%) 13th day	Root length 14th day	Leaf length 14th day
Injured				
Check	92	88	205	87
Formaldehyde	72	42	94	39
Ceresan	96	90	256	96
Nomersan	94	82	187	79
Spergon	96	88	195	86
Uninjured				
Check	100	90	278	86
Formaldehyde	94	72	130	61
Ceresan	96	94	257	101
Nomersan	100	88	250	85
Spergon	98	94	249	103
Min. sig. diff.	-	1.5	71.4	21.1

TABLE XXII

Analysis of variance of data on germination and emergence from Table XXI

		Mean squares		
		Germination		Emergence
Variance due to	D.F.	3rd day	14th day	13th day
Replicates	4	1012	222	177
Treatments	4	1697*	367	2232**
Injury	1	3362*	722	1152**
Injury x Treat.	4	447	187	332
Error	36	499	293	138
Total	49			

* Significant to the 5% point
** Significant to the 1% point

The daily germination records indicate that of the five treatments on injured and uninjured wheat only the formaldehyde on the injured seed appreciably reduces the rate of germination. The differences on the third day after planting are significant, but these tend to disappear and, by the 14th day, they are no longer apparent. Moreover artificial injury to the embryo covering actually caused little difference in the final number of germinated seeds. In spite of this the differences in emergence are highly significant. With injured seed only Ceresan treatment increases emergence, while formaldehyde and Nomersan lower it. With uninjured normal seed both Ceresan and Spergon show marked benefit while formaldehyde and Nomersan again lower the emergence significantly.

Obviously all the seeds which germinate do not emerge. The conclusion is that between germination and emergence death has resulted, and therefore the difference in germination and emergence of the same plants should give some index of the value of the treatment. These differences are shown in Table XXIII.

TABLE XXIII

Percent germinated seeds failing to produce emerged seedlings after 13 days

Treatment	Germination	Emergence	Difference
Injured			
Check	92	88	4
Formaldehyde	72	42	30
Ceresan	96	90	6
Nomersan	94	82	12
Spergon	96	88	8
Uninjured			
Check	100	90	10
Formaldehyde	94	72	22
Ceresan	96	94	2
Nomersan	100	88	12
Spergon	98	94	4

Artificial injury tends to have an over all detrimental effect on both germination and emergence. It is apparent from the above table that in the case of uninjured seed, Ceresan and Spergon produce the most beneficial results in so far as emergence is concerned. With injured seed the same trend is shown, though the checks do better than might

be expected, probably because the broken covering permits quicker absorption of water, allowing an earlier start for the seedling.

Formaldehyde greatly slows down the rate of growth and, while the percentage germination is not markedly low, especially when the seed is uninjured, the emergence tends to be low and the percentage of the non-surviving seedlings is very high.

The explanation of these results is considered to lie with certain soil sarophytes, mainly Penicillium and Mucor spp. and bacteria. The time that the seed showed evidence of attack was noted and the per cent daily attack of the wheat seedlings by soil saprophytes is tabulated in Table XXIV.

TABLE XXIV

Percent of seeds attacked by soil saprophytes recorded at daily intervals from the sixth to the fourteenth day

Treatment	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Injured									
Check	6	14	18	20	22	22	26	26	26
Formaldehyde	24	36	40	40	42	50	60	66	70
Ceresan	4	4	4	4	4	4	6	6	8
Nomersan	14	22	26	30	30	34	36	36	40
Spergon	8	16	26	30	32	42	42	46	46
Uninjured									
Check	16	22	26	26	28	28	36	36	38
Formaldehyde	8	20	26	30	40	40	44	44	48
Ceresan	0	2	4	4	4	8	16	16	16
Nomersan	4	14	16	16	18	24	24	24	26
Spergon	0	6	10	14	14	16	24	24	24

The results with injured seed are especially interesting. With the formaldehyde treatment the slowing down of the rate of growth so encouraged attack by soil organisms that in two weeks 70% of the seeds and seedlings from injured seed were affected. This of course does not mean that all were necessarily killed, as many seeds were attacked after the seedlings emerged. Many of the latter were stunted, as shown by the leaf data. The Ceresan treatment on the other hand gave a high degree of protection, only 8% of the seeds showing attack. Neither Spergon nor Nomersan show much protective action and, in fact, both seem to encourage the growth of microorganisms under the conditions of this experiment.

With normal seed, formaldehyde again tends to encourage mold and bacterial growth, while all the other treatments tend to exert some protective effect, the greatest amount again being shown by Ceresan.

TABLE XXV

Analysis of variance for leaf length and root growth on the 14th day as shown in Table XXI

Variance due to	D.F.	Mean squares: Leaf length	Mean squares: Root length
Replicates	4	729	6603
Injury	1	1172*	21757*
Treatment	4	3665**	31171**
Injury x Treat.	4	230	2316
Error	36	285	3262
Total	49		

* Significant to the 5% point
** Significant to the 1% point

Injury to the seed resulted in a significant decrease in leaf length of seedlings from seed treated with formaldehyde, while it resulted in a significant decrease of root growth only in the case of the checks.

The effect of seed treatment on leaf growth and root growth was significantly detrimental only in the case of formaldehyde treatment on both injured and uninjured seed. Ceresan gave indications of benefit on both injured and uninjured seed, while Spergon appeared to benefit normal seeds to a greater extent than injured seed.

Experiment with Oats

An experiment was made with Victory oats to determine whether the effect of injury and seed treatment was similar to that for wheat. The hulls were removed, and a portion of the seed-lot was artificially injured as in the previous experiment, using a needle. The seed was treated with formaldehyde, Ceresan, and Spergon as in the preceding experiment and sown in the glass-fronted containers at the rate of 25 seeds per can.

Results

The results are summarized in Table XXVI and the daily growth of the roots is recorded in Appendix XI. The figures for the formaldehyde treatments were so low that they were not included in the analysis of variance shown in Table XXVII.

TABLE XXVI

Summary of experiment on seed treatment of injured and uninjured oats+

Treatment	Emergence (%)	Root length* 14th day	Leaf length* 14th day
Injured			
Check	27	296	44
Formaldehyde	0	6	0
Ceresan	46	270	68
Spergon	55	405	78
Uninjured			
Check	65	387	124
Formaldehyde	13	33	16
Ceresan	84	558	168
Spergon	72	428	139
Min. sig. diff.**	11.6	60.2	18.8

+ Hulls removed
* Average per container
** Formaldehyde not included in analysis

TABLE XXVII

Analysis of variance of data in Table XXVI

		Mean square		
Variance due to	D.F.	Emergence	Leaf length	Root length
Replicates	3	147	372	1750
Injury	1	5828**	38962**	108004**
Treatments	2	918**	2546**	14589*
Injury x Treat.	2	301	751	37677**
Error	15	129	355	3628
Total	23			

*Significant to the 5% point
**Significant to the 1% point

NOTE: The formaldehyde results were not included.

The emergence analysis indicates that the variability due to seed injury is very high with all treatments. Formaldehyde markedly lowered emergence of both injured and uninjured seed but Ceresan and Spergon greatly increased it. A similar effect in general was shown by the leaf length and root length data. In the case of the latter the damage-treatment interaction indicates that the treatments affect damaged and undamaged seed differently. The same trend, while not significant, is evident in the case of the emergence and leaf length figures. This is apparently due to the fact that Ceresan exerts a detrimental effect on injured seed and a beneficial effect on normal seed, while Spergon shows a greater increase than Ceresan with injured seed but a lesser increase with uninjured seed.

Effects of Seed Treatment of Injured and Uninjured Seed in Sterilized and Unsterilized Soil

The claim has been made that some of the beneficial effects of certain seed treatments result from chemical stimulation of the seed. However, under natural dconditions the effect on the seed itself is not readily separated from that on the soil microflora. If a definite increase can be shown to be due to the chemical in the absence of soil microorganisms, this claim may have some foundation.

Method

Glazed six-inch pots were filled with 1400 grams of air-dry 3:1 soil-sand mixture, moistened with 330 cc. of water covered with newspaper and sterilized for 15 hours at 15 pounds pressure. After seeding 25 seeds per pot, 320 grams of sterile soil was placed on top. A similar series unsterilized was used as a control. The pots were then watered.

The seed used was of the Red Bobs variety. Each kernel was examined to ensure that the only difference between a normal seed and an injured seed was the broken embryo covering of the latter. Seeds with cracked endosperms were discarded. Only about 25% of the seeds in the sample had damaged coverings, so the deficiency was made up by artificial injury as in the previous experiment. The seed was treated in the usual manner.

To determine the effect of treatment on the rate of emergence, counts were made at four-hour intervals for three days and at 12- and 24-hour intervals for some time later. These data are recorded in Appendix VIII. After 14 days of growth, the plants were carefully washed free of soil and the top and root lengths were measured to the nearest inch. They were then freed of the old seed, dried, and the dry weight was determined.

Results

The results of the experiment are summarized in

Table XXVIII. The rate of emergence data are recorded in Appendix VIII and graphically illustrated in Figure 12. The analysis of variance for root and leaf data are shown in Tables XXIX, XXXI, and for emergence in Table XXX.

TABLE XXVIII

Effect of seed treatment of injured and uninjured wheat seed in sterilized and unsterilized soil

Seed	Treatment	Emergence (%) 10th day	Roots* Length	Roots* Weight	Leaves* Length	Leaves* Weight
Sterilized soil						
Injured	Check	93.5	289	.09	220	.36
	Formaldehyde	73.0	210	.07	131	.28
	Ceresan	97.5	303	.09	206	.41
	Nomersan	95.5	338	.12	225	.48
	Spergon	91.5	284	.08	187	.36
Uninjured	Check	94.0	328	.11	230	.45
	Formaldehyde	92.5	266	.09	216	.44
	Ceresan	98.5	327	.10	218	.44
	Nomersan	96.0	316	.10	228	.46
	Spergon	93.0	317	.10	216	.41
Unsterilized soil						
Injured	Check	82.0	295	.08	135	.24
	Formaldehyde	40.5	134	.04	60	.16
	Ceresan	89.5	341	.12	188	.34
	Nomersan	86.5	243	.06	131	.21
	Spergon	90.0	449	.12	182	.32
Uninjured	Check	90.0	324	.10	156	.26
	Formaldehyde	66.0	186	.05	150	.19
	Ceresan	97.5	473	.16	207	.42
	Nomersan	92.5	334	.11	168	.30
	Spergon	98.5	518	.15	214	.40
Minimum significant difference		6.5	94	.09	33	.11

* Measurements on 18th day, average per container; lengths in inches, weights in grams.

TABLE XXIX

Analysis of variance of data in Table XXVIII

		Mean square			
		Root		Leaf	
Variance due to	D.F.	length	weight	length	weight
Treatments in general	19	32909**	338**	80427**	3826**
Replicates	3	1863	27**	1003	443
Error	57	4521	5	5642	570
Total	79				

* Significant to 5% point
** Significant to 1% point

TABLE XXX

Analysis of variance for emergence data

Variance due to	D.F.	MS
Treatments in general	19	1578**
Replicates	7	82
Error	133	42
Total	159	

** Significant to 5% point

TABLE XXXI

Analysis for variance for root length on 18th day

Variance due to	D.F.	MS
Soil	1	20353
Replicates	3	1863
Error (1)	3	11080
Injury	1	51006
Injury x soil	1	11663
Error (2)	6	10924
Treatments	4	86409
Treat. x Injury	4	1290
Treat. x soil	4	44330**
Treat. x Injury x Soil	4	3537
Error (3)	48	2012
Total	79	

The treatments in general show significant differences in emergence and in the lengths and weights of the roots and leaves. When the data are broken up, these differences in the case of root growth are shown to be due to the interaction between treatments and soil. That is in the unsterilized soil the chemical treatments have a different effect than they have in sterilized soil. This is especially apparent in the case of the formaldehyde.treatment which is illustrated in Figure 15. The effect of Ceresan, while markedly beneficial in the case of emergence and in root and leaf growth in unsterilized soil, shows little of this benefit in sterilized soil. Sterilization of the soil generally improved the

seedling growth of untreated seed, but the use of Ceresan brought the level of the growth in unsterilized soil to that of the check in sterilized soil. This is illustrated in Figure 14.

A comparison of normal wheat seed with seed having the covering broken shows that the emergence was not appreciably lowered by any of the treatments. The emergence, however, was impaired in certain cases. Not all of the seed that germinated was able to survive and send seedlings above the soil surface. This is well illustrated by the formaldehyde treatment. In sterilized soil uninjured wheat treated with this chemical was equally as good as that of the checks (Figure 12, lower left) but in unsterilized soil the damage was very great in spite of the fact that the germination had been 94%. Obviously, between the times of germination and emergence, many of the seedlings were killed, presumably by the attack of soil organisms.

It would appear that the protective action to seed of chemicals could be evaluated by comparing the differences between the emergence and germination figures. On this basis Ceresan appears to have a high protective action on both injured and uninjured seed, Spergon on uninjured seed, and formaldehyde on neither injured nor uninjured seed.

Moreover, injured seed might be expected to be attacked by soil saprophytes more than uninjured, and this generally held for the formaldehyde, Nomersan, and Spergon treated seed. However, the injured seed checks showed far

seedling growth of untreated seed, but the use of Ceresan brought the level of the growth to [illegible] of the check in sterilized soil. This is illustrated in Figure 14.

A comparison of normal check seed with seed having the intervening brown spots shows that the germination was appreciably lowered by any of the treatments. The emergence, however, was lowered in [illegible]. Not all of the seed that germinated was able to survive and send seedlings above the soil surface. This is well illustrated by the [illegible] treatment. In sterilized soil [illegible] seed treated with this chemical was [illegible] as good as seed of [illegible] (Figure 15, lower left) but in unsterilized soil the [illegible] was very great in spite of the fact that the germination was [illegible]. Obviously, between the times of germination and emergence, many of the seedlings were killed, presumably by the attack of soil organisms.

It would appear that the protective action of some of chemicals could be evaluated by comparing the difference between the emergence and germination figures. On this basis Ceresan appears to have a high protective action on both diseased and uninfected seed, [illegible], and formaldehyde on [illegible] seed.

Moreover, [illegible] seed might be expected to be attacked by soil saprophytes more [illegible] especially valid for the formaldehyde [illegible], and [illegible] treated seed. However, the [illegible]

less mold damage than normal seed, possibly owing to a quicker utilization of the soil moisture because of the broken seed coats. The Ceresan treated injured seed also showed little mold damage owing to a protective action by the chemical or to a stimulation which promoted faster growth of the plant.

In the opinion of Popoff (38) the stimulation of the organic mercury dusts is not restricted to the period of germination but is carried on to the close of vegetation. The existence of such a stimulation is not generally accepted (23) but the appreciable increases in emergence over the checks, due to Ceresan on both injured and uninjured wheat and to Nomersan in injured wheat in sterilized soil, where presumably no saprophytes are present, indicates some stimulation by the chemicals. This increase, due to Ceresan, is not carried into the leaf and root data. That due to Nomersan is apparent in the root growth but only with injured seed in sterilized soil. In the case of Spergon the stimulation reported by Garrow (21) and McNew (36) for peas seems entirely absent with wheat. (See Figure 13, upper left).

The indications are that in unsterilized soil both Ceresan and Spergon have a marked beneficial effect especially upon uninjured seed. Spergon tends to give an earlier start in emergence but is surpassed by Ceresan in sterilized soil. (See Appendix VIII). In unsterilized soil Spergon treated seed shows the greatest root length (Figure 13). Formaldehyde treatment consistently had a detrimental effect through-

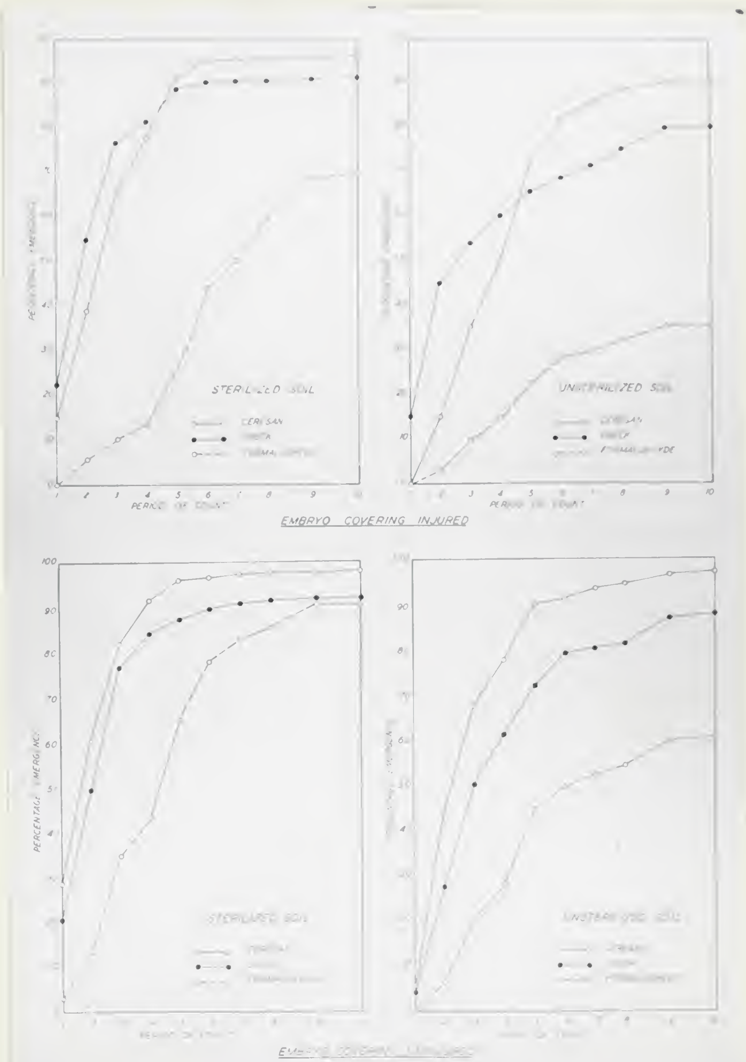


Figure 12

The effect of seed treatment on the rate of emergence of injured and uninjured wheat in sterilized and unsterilized soil. Periods 1 to 8 were at 8-hour intervals, 8 to 10 at 12-hour intervals.

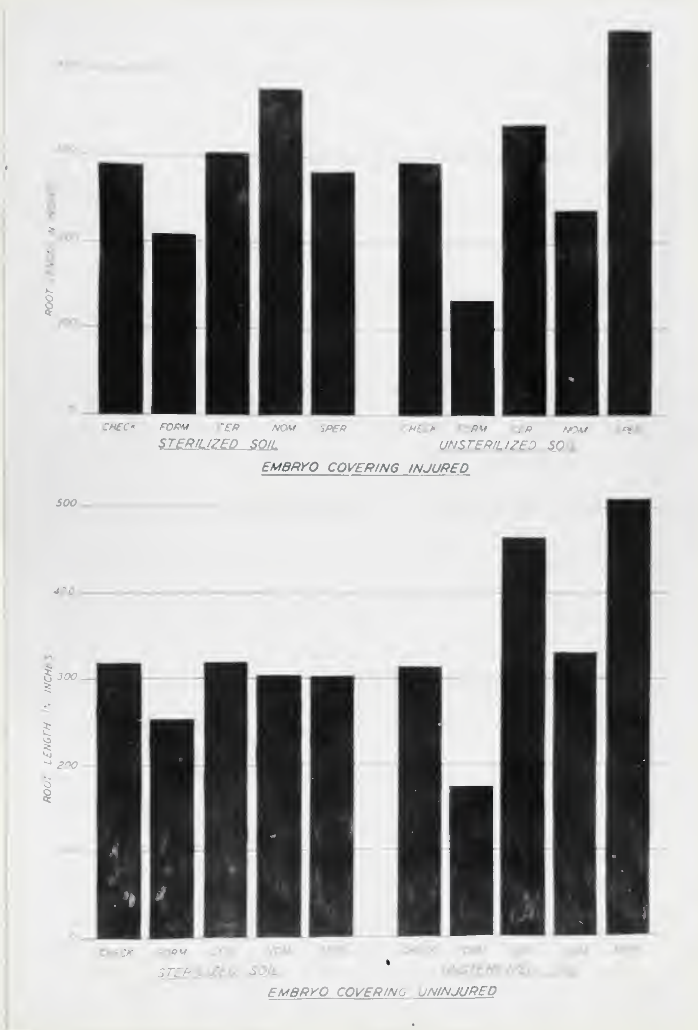


Figure 13

The effect of seed treatment on the root growth of injured and uninjured wheat in sterilized and unsterilized soil. Measurements were taken on the 14th day after seeding.

Unsterilized soil Check | Unsterilized soil Ceresan | Sterilized soil Check

Figure 14

The improvement by Ceresan treatment of uninjured wheat growing in unsterilized soil.

Unsterilized soil Formaldehyde | Sterilized soil Formaldehyde

Figure 15

The relative amount of injury by formaldehyde seed treatment of wheat in sterilized and unsterilized soil.

out the experiment.

Discussion

Seed treatment may have two main effects on clean seed: (1) a direct effect on the host, either stimulatory or phytocidal; (2) a protective action against soil saprophytes.

The increase in emergence due to treatment in sterilized soil where no soil saprophytes are present is attributed to a stimulatory effect by the chemical. Indications of this are shown only by the Ceresan treatment among those tested.

Ceresan however may also have severe phytocidal effects if it is applied to moist seed. This is clearly illustrated by the experiment with inoculated hulled and hulless oats (Page 19). A severe phytocidal effect is also shown when dehulled oats with the pericarp broken are treated with Ceresan. The symptoms here are typical of Ceresan injury as reported by Sass (45), including swollen roots and leaves accompanied by stunted growth. The protective action of Ceresan against soil saprophytes is well demonstrated by the marked decrease in mold damage. Thus it is apparent that, with this chemical, there is a fine balance between damage and benefit.

No phytocidal effect by Spergon was demonstrated. The stimulatory action reported on peas (36) was not apparent. in the case of wheat. Under natural conditions, however, with

unsterilized wheat and uninjured seed Spergon treatment showed a marked beneficial effect, indicating a highly effective protection against soil saprophytes. It is noted, however, that this protection is lessened under conditions of excessive soil moisture. Under such condtions, the attack by molds and other organisms does not appear to be checked.

Formaldehyde treatment appears to slow down the rate of growth definitely (Figure 12). With wheat, decreases in emergence often occur. In the absence of soil microorganisms, however, this decrease is very small either with injured or uninjured seed. The explanation is that in sterilized soil the soil saprophytes that normally would gain ready entrance through damaged seed coats have been killed. Growth is only slightly impaired by the seed injury, but though this injury in itself is not harmful, in unsterilized soil in the presence of the soil microorganisms, marked ill effects are caused. (Figure 16).

The justification for treating clean seed with Ceresan as a routine procedure is that the emergence and yield are increased (23). On the other hand, Greaney and Machacek (24) state that unless an appreciable amount of contamination is present, treatment of the seed is unnecessary.

The environmental conditions such as soil type, pH, microorganic flora, undoubtedly have some bearing on the value of seed treatment. For example Henry and Ludwig (57) have shown that seed treatment of peas with Ceresan may be of greater value in a brown soil than in a sandy soil.

Ceresan

Formaldehyde

Check

Figure 16

The effect of seed treatment on hulless Victory oats.

It appears that the improvement to wheat by Spergon treatment is primarily due to a protective action against soil microorganisms, while that by Ceresan is due to an early short period of stimulation to the germinating seed, in addition to a protective action. Formaldehyde, on the other hand, appears to have a phytocidal and non-protective action.

It should be noted that the improvement due to seed treatment cannot be wholly judged by such characters as root length and leaf growth. The improvement in health and stand is often apparent to the eye but difficult to tabulate. This is illustrated in Figure 16 where hulless oats treated with Ceresan is compared with that treated with formaldehyde and untreated checks.

SUMMARY

1. Halo Blight of oats occurs widely in Alberta. It probably causes more damage than is attributed to it.

2. *Bacterium coronafaciens*, the causal organism of Halo Blight, was isolated from infected leaves and heads of Erban oats from an Alberta field.

3. Alberta isolates of *B. coronafaciens* were found to agree in essential characters with pure cultures of this organism. When artificially inoculated on Victory oats they proved to be pathogenic.

4. Twenty-nine varieties of oats artificially inoculated all proved susceptible in varying degrees to local isolates of the pathogen.

5. Oat seed inoculated with *B. coronafaciens* produced seedlings showing symptoms of primary infection. The following were the main effects: (1) prevention of seed from germinating; (2) blighting of young seedlings; (3) lesioning of the first leaves.

6. The soil temperature range for primary infection was found to be 14°C to 28°C.

7. Seed treatment with neither Ceresan, Spergon nor formaldehyde completely prevented infection of oat seedlings from

seed contaminated with *B. coronafaciens*.

8. Certain bacteria isolated from soil and plant tissue were highly antagonistic to *B. coronafaciens* in culture media. These were superior to antagonists obtained from other sources for comparison.

9. Some of the above mentioned antagonists, when applied to oat seeds contaminated with *B. coronafaciens*, reduced the severity of seedling infection.

10. A fungal antagonist, namely *Penicillium* Ac, applied to contaminated oats, also reduced the severity of primary infection.

11. Formaldehyde treatment caused severe injury, especially to hulless oats and damaged wheat, owing to retarding the growth rate and predisposing the seed to attack by soil saprophytes.

12. Ceresan had a slight stimulatory effect on wheat as measured by seedling emergence. It was highly effective in protecting the seed against attack of soil saprophytes. The treatment showed more benefit to uninjured seed than to injured seed.

13. Nomersan stimulated wheat seedling emergence and caused a marked increase in root growth with injured seed in sterilized soil. It had little protective action against soil saprophytes.

14. Spergon increased the rate of emergence and had a beneficial effect on clean seed oats and wheat. It was not effective against soil saprophytes in soil with a high moisture content.

15. Mechanical injury to oat and wheat seed was not great in itself. The detrimental effects appeared to be due to increased attack by soil saprophytes.

16. Both Spergon and Ceresan applied to clean uninjured seed of wheat and oats tended to increase the emergence, leaf and root growth. The greatest response was obtained in unsterilized soil, which suggests that under natural field conditions such treatments should have beneficial effects.

ACKNOWLEDGEMENTS

The writer wishes to express his thanks to Dr. A. W. Henry, Associate Professor of Plant Pathology, University of Alberta, under whose direction this work has been carried out, for his helpful suggestions and criticisms during the progress of this investigation; to Dr. A. G. McCalla for his assistance in connection with the statistical analysis; and to the Canadian Industries Limited and the Bayer-Semesan Company for financial assistance.

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APPENDIX I

Effect of seed treatment with formaldehyde and Ceresan on the root growth of hulled and hulless Victory oats inoculated with oat blight isolates

		Daily root length in inches*											
Hull	Treatment	1	2	3	4	5	6	7	8	9	10	11	12
T 33b													
Hulled	Form. 1:320	49.3	63.3	117.8	175.5	244.0	272.8	301.5	311.3	357.3	385.0	414.8	436.5
	Ceresan ½ oz.	39.0	69.0	132.0	165.5	220.5	264.8	301.8	342.0	354.5	378.5	400.5	425.0
	Check	32.8	70.3	116.0	161.3	218.5	260.5	287.0	329.0	347.3	360.0	383.3	430.0
Hulless	Form. 1:320	9.0	15.8	20.3	26.5	31.0	36.3	41.0	44.5	44.0	47.0	49.5	51.0
	Ceresan ½oz.	12.0	20.8	26.8	38.3	54.0	64.3	71.3	73.0	79.0	86.5	87.5	95.3
	Check	17.8	30.5	56.5	84.8	114.8	131.0	148.3	164.8	167.0	183.5	199.3	211.3
T 37													
Hulled	Form. 1:320	34.8	61.5	96.0	163.3	225.5	282.0	324.5	365.3	378.5	411.3	436.5	473.0
	Ceresan ½oz.	35.8	64.0	110.0	156.3	201.5	264.3	302.0	325.3	367.0	395.8	421.8	455.8
	Check	28.0	48.3	103.3	150.3	229.8	299.8	333.8	373.3	390.3	413.5	451.0	476.5
Hulless	Form. 1:320	11.3	16.0	31.8	43.8	57.8	74.5	92.0	110.3	121.5	126.5	132.8	141.5
	Ceresan ½oz.	20.3	29.5	53.8	77.5	104.8	140.0	156.8	172.5	186.5	223.0	240.3	256.8
	Check	19.8	40.0	70.5	107.5	156.5	209.0	225.5	257.8	276.3	292.5	313.3	335.0
Control													
Hulled	Form. 1:320	41.0	74.8	122.0	177.8	242.5	306.5	351.5	373.3	397.8	416.0	450.3	474.0
	Ceresan ½oz.	41.8	65.5	121.5	184.0	253.5	314.5	342.0	364.3	414.0	440.0	489.3	533.5
	Check	44.3	77.5	132.0	203.5	285.3	353.0	398.5	422.8	458.3	485.0	528.8	559.0
Hulless	Form. 1:320	12.3	19.0	36.0	46.5	58.0	78.0	86.8	98.3	117.3	123.0	134.0	145.8
	Ceresan ½oz.	31.3	42.5	74.3	103.8	142.3	163.3	180.8	200.0	207.0	224.8	232.8	247.8
	Check	32.5	58.5	94.5	130.8	192.0	226.5	272.5	291.3	311.0	336.0	360.5	388.3

* Average per container

APPENDIX II

Daily root growth of Victory oats after inoculation with B. coronofaciens and treatment with formaldehyde, Ceresan, and Spergon

	Daily root length in inches*								
Treatment	1	2	3	4	5	6	7	8	9
Inoculated									
Check	97	193	334	418	526	581	624	669	688
Formaldehyde 1:320	89	194	280	389	493	557	605	635	665
Ceresan $\frac{1}{2}$ oz.	98	198	327	412	478	548	603	690	734
Spergon 2 oz.	88	193	279	378	438	500	559	609	658
Not inoculated									
Check	110	210	350	453	597	628	701	751	810
Formaldehyde 1:320	102	201	304	383	460	531	594	645	679
Ceresan $\frac{1}{8}$ oz.	97	197	350	452	524	623	682	771	851
Spergon 2 oz.	111	220	337	403	470	549	620	699	765

* Average per container

APPENDIX III

Daily root growth of Victory oats inoculated with _B_. _coronofaciens_ and treated with antagonistic bacteria

	Daily root length in inches*									
Treatment	1	2	3	4	5	6	7	8	9	10
Inoculated										
Ck	79	122	178	211	263	305	343	378	403	423
A8	65	114	166	210	232	289	310	347	372	398
A9	79	129	176	235	304	347	387	409	452	487
A10	77	116	170	219	286	331	360	389	415	437
A42	74	117	183	221	270	318	368	403	473	503
Not inoculated										
Ck	102	147	214	287	351	385	400	432	448	464
A8	112	157	204	265	331	384	420	457	494	529
A9	99	146	240	304	356	402	430	462	497	540
A10	140	184	241	301	340	385	425	456	481	497
A42	100	168	237	282	344	404	454	499	536	568

* Average per container

APPENDIX IV

Daily root growth of barley, oats, and rye after seed treatment with Ceresan and formaldehyde

	Daily root length in inches*											
Treatment	1	2	3	4	5	6	7	8	9	10	11	12
<u>Barley</u>												
Ceresan ½oz.	53.5	63.3	75.6	75.4	74.7	90.4	84.5	88.4	94.4	98.3	137.5	152.4
Form. 1:320	51.0	60.7	66.8	65.8	74.5	84.8	80.4	92.2	104.0	101.1	139.1	161.7
Check	53.2	61.0	82.7	88.9	80.2	81.6	93.1	97.9	107.6	108.8	146.6	183.3
<u>Oats</u>												
Ceresan ½oz.	21.5	42.0	67.9	105.0	169.8	200.6	196.3	233.7	263.1	273.0	332.1	380.4
Form. 1:320	15.8	27.7	41.4	61.5	80.8	95.1	115.3	122.4	126.5	155.6	176.3	197.6
Check	22.7	44.9	78.0	102.3	160.9	190.9	203.3	217.4	213.4	245.7	325.6	337.4
<u>Rye</u>												
Ceresan ½oz.	26.3	44.8	58.0	68.0	76.5	98.3	92.4	106.9	122.7	103.3	125.3	178.8
Form. 1:320	5.1	7.3	9.8	14.7	15.2	16.5	22.1	22.8	24.8	36.2	32.8	34.5
Check	22.2	35.6	45.2	51.7	60.6	71.7	78.7	84.9	95.7	99.2	118.6	130.2

* Average per container

APPENDIX V

Daily root growth of clean oats and wheat treated with Ceresan at three different rates

Treatment	Daily root length in inches*								
	1	2	3	4	5	6	7	8	9
Victory oats									
Ceresan 0	114	167	226	354	481	550	614	672	725
1/4 oz.	106	152	211	298	455	551	641	694	794
1/2 oz.	95	167	213	305	444	555	602	670	753
1 oz.	89	131	213	291	398	512	616	670	719
Red Bobs wheat									
Ceresan 0 oz.	43	152	198	226	290	315	343	370	403
1/4 oz.	55	165	250	265	315	364	386	410	439
1/2 oz.	47	157	217	260	305	332	352	380	409
1 oz.	40	142	178	237	300	348	374	410	438

* Average per container

APPENDIX VI

Effect of seed treatment on the daily root growth of injured and non-injured Red Bobs wheat

Treatment	Daily root length in inches*													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Injured seed														
Check	8.8	30.2	44.3	61.6	86.7	109.4	132.5	151.1	155.6	162.6	168.9	182.8	200.9	205.0
Form. 1:320	2.4	10.3	16.5	22.7	35.9	52.0	62.0	64.5	68.2	71.9	76.6	80.7	86.9	94.2
Ceresan $\frac{1}{3}$oz.	6.6	25.1	36.5	56.1	81.0	111.7	133.7	158.8	178.2	187.8	195.7	211.4	228.2	256.3
Nomersan 2oz.	8.5	27.1	40.2	57.6	73.7	97.8	120.6	127.5	138.8	144.6	150.6	153.3	167.2	187.2
Spergon 2oz.	7.4	25.2	35.2	53.0	78.9	102.5	117.5	130.1	135.0	138.4	146.7	162.3	183.2	195.2
Non-injured seed														
Check	7.6	30.2	48.1	69.1	92.9	122.5	158.6	170.2	182.2	192.8	200.4	210.6	250.5	277.5
Form. 1:320	4.3	18.0	26.4	37.2	53.4	72.9	90.6	94.0	101.2	105.8	108.0	114.6	118.5	129.7
Ceresan $\frac{1}{3}$ oz.	7.9	30.0	47.0	68.3	88.1	118.7	152.5	168.1	180.6	190.5	198.9	209.2	238.3	256.8
Nomersan 2oz.	5.8	28.5	40.9	60.4	92.9	127.6	159.8	189.6	199.4	207.8	218.4	229.4	241.8	250.4
Spergon 2oz.	13.0	44.5	61.3	82.4	117.0	141.5	157.5	171.1	183.4	188.7	196.1	202.8	232.1	248.6

* Average per container

APPENDIX VII

Effect of seed treatment on the daily leaf growth of injured and non-injured Red Bobs wheat

	Daily leaf length in inches*												
Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13
Injured seed													
Check	2.0	4.4	10.5	20.2	32.3	41.9	50.6	57.0	61.1	64.7	70.9	76.2	86.6
Form. 1:320	0.3	0.6	2.1	4.2	10.9	17.3	21.8	25.7	28.2	30.1	31.5	33.4	38.8
Ceresan ½ oz.	1.0	2.5	6.0	17.3	30.6	44.8	56.4	64.5	67.6	71.3	75.1	86.4	96.4
Nomersan 2 oz.	0.9	1.8	6.7	17.3	26.8	39.9	48.8	54.8	57.3	60.9	64.2	71.6	78.8
Spergon 2 oz.	0.9	2.5	7.0	17.4	30.9	46.0	55.5	61.3	63.8	67.0	70.4	78.0	85.5
Non-injured seed													
Check	0.8	1.8	5.8	17.7	30.0	43.7	50.8	61.4	63.2	65.4	68.8	74.2	85.9
Form. 1:320	0.4	0.8	2.8	10.1	21.2	31.6	39.2	46.3	49.8	51.3	52.4	56.3	61.2
Ceresan ½ oz.	0.8	2.8	8.5	21.2	36.1	55.2	64.6	73.1	77.0	81.2	82.8	90.7	100.3
Nomersan 2 oz.	0.9	1.8	6.6	16.5	28.2	43.0	54.0	62.1	65.3	67.1	69.8	76.2	85.0
Spergon 2 oz.	1.1	2.6	11.6	22.7	37.0	32.5	63.1	69.3	72.0	73.9	80.8	91.5	102.6

* Average per container

APPENDIX VIII

Effect of seed treatment on the rate of emergence of injured and uninjured wheat growing in sterile and unsterile soil

Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Sterile																							
Injured seed																							
Check	24.5	40.0	55.5	73.0	77.5	78.5	82.5	83.5	88.5	90.5	90.5	90.5	90.5	91.0	91.5	91.5	92.0	92.5	92.5	92.5	93.0	93.0	93.5
Form. 1:320	0.0	3.5	5.5	6.5	10.5	10.5	13.5	18.5	29.5	39.5	44.0	46.0	50.5	54.0	60.5	64.0	68.0	69.0	70.5	71.0	71.5	72.0	73.0
Ceresan ½ oz.	15.0	24.5	38.0	61.5	65.0	67.0	77.5	82.5	90.5	95.0	95.0	95.0	95.5	95.5	95.5	96.0	97.0	97.5	97.5	97.5	97.5	97.5	97.5
Nomersan 2 oz.	27.0	42.0	53.0	71.0	76.5	78.0	81.0	86.0	90.5	92.5	92.5	92.5	92.5	93.5	93.5	93.5	95.0	95.0	95.5	95.5	95.5	95.5	95.5
Spergon 2 oz.	20.0	31.0	57.0	63.0	69.0	70.0	76.5	79.5	86.5	88.5	88.5	89.0	89.0	89.0	89.0	89.0	91.0	91.0	91.5	91.5	91.5	91.5	91.5
Uninjured seed																							
Check	21.0	34.5	50.5	70.0	77.0	78.5	85.5	86.5	88.5	90.5	91.5	92.0	92.0	92.0	92.0	92.0	92.5	92.5	93.5	93.5	93.5	94.0	94.0
Form. 1:320	3.5	6.0	13.5	26.0	35.5	37.0	43.5	53.5	66.5	73.5	78.5	81.0	82.5	82.5	84.5	86.0	90.5	91.0	92.0	92.0	92.0	92.5	92.5
Ceresan ½ oz.	29.5	48.0	61.5	75.5	83.5	84.5	92.0	94.0	97.0	97.5	97.5	97.5	97.5	97.5	98.0	98.0	98.5	98.5	98.5	98.5	98.5	98.5	98.5
Nomersan 2 oz.	16.0	32.0	52.0	68.0	74.5	77.0	83.5	86.0	90.0	93.0	93.5	94.0	95.0	95.0	95.0	95.0	95.0	95.0	95.5	95.5	96.0	96.0	96.0
Spergon 2 oz.	41.0	60.5	73.0	83.0	87.5	87.5	89.5	90.5	91.5	92.0	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5	93.0	93.0	93.0
Unsterile																							
Injured seed																							
Check	16.5	32.0	44.0	51.0	53.0	55.0	57.0	61.0	66.0	67.0	68.5	70.5	72.0	72.5	75.5	75.5	79.0	79.5	79.5	81.0	81.5	82.0	82.0
Form. 1:320	0.0	1.0	3.0	9.5	9.5	10.5	15.0	16.5	23.5	26.0	28.0	29.5	29.5	30.0	33.0	36.0	36.0	37.0	37.5	37.5	39.0	39.5	40.5
Ceresan ½ oz.	0.0	5.0	15.5	33.0	36.0	40.0	50.5	59.5	73.0	80.0	82.0	84.0	86.0	87.0	88.0	88.5	89.5	89.5	89.5	89.5	89.5	89.5	89.5
Nomersan 2 oz.	14.0	27.0	40.5	55.5	60.5	61.0	64.5	70.0	74.5	77.5	79.5	80.0	80.5	82.0	82.5	82.5	84.0	84.0	84.0	84.5	85.5	85.5	86.5
Spergon 2 oz.	16.0	36.5	27.5	44.5	51.5	54.5	62.5	67.5	74.0	76.5	79.0	79.0	81.0	83.0	83.5	84.5	87.5	88.0	88.0	89.0	90.0	90.0	90.0
Uninjured seed																							
Check	4.0	15.5	27.5	45.5	51.5	54.5	62.5	71.5	74.0	76.5	79.0	79.0	81.0	83.0	83.5	84.5	87.5	88.0	88.0	89.0	90.0	90.0	90.0
Form. 1:320	0.0	5.5	7.5	16.0	21.0	23.5	28.0	38.5	45.5	48.0	49.5	50.5	52.0	54.5	55.5	57.0	61.0	62.5	64.5	65.0	65.5	66.0	66.0
Ceresan ½ oz.	5.5	26.5	43.0	63.0	68.5	72.5	78.5	85.5	91.0	92.5	93.0	95.0	95.5	96.0	96.5	97.0	97.5	97.5	97.5	97.5	97.5	97.5	97.5
Nomersan 2 oz.	6.5	27.0	41.0	51.0	53.0	57.0	64.5	73.0	80.5	85.0	85.5	87.5	88.5	89.0	90.5	91.5	92.0	92.0	92.0	92.0	92.5	92.5	92.5
Spergon 2 oz.	21.0	43.5	60.5	75.0	80.5	81.5	86.5	93.0	94.0	96.5	96.5	97.0	97.5	98.0	98.0	98.0	98.5	98.5	98.5	98.5	98.5	98.5	98.5

Counts 1 - 16 at 4-hour intervals
Counts 17 - 21 at 8- and 12-hour intervals, alternately
Counts 22 - 23 at 24-hour intervals.

APPENDIX IX

Effect of seed treatment on the percentage germination of injured and non-injured wheat

	Percent daily germination											
Treatment	1	2	3	4	5	6	7	8	9	10	11	12
Injured												
Check	84	90	90	90	90	92	92	92	92	92	92	92
Form. 1:320	40	62	68	68	68	70	70	70	70	70	70	72
Ceresan ½oz.	68	90	96	96	96	96	96	96	96	96	96	96
Nomersan 2oz.	80	84	86	90	92	92	92	94	94	94	94	94
Spergon 2oz.	78	88	92	94	94	94	94	96	96	96	96	96
Non-injured												
Check	94	98	98	98	98	98	100	100	100	100	100	100
Form. 1:320	72	84	88	88	88	88	88	88	90	92	94	94
Ceresan ½oz.	94	94	94	96	96	96	96	96	96	96	96	96
Nomersan 2oz.	78	94	98	100	100	100	100	100	100	100	100	100
Spergon 2oz.	94	98	98	98	98	98	98	98	98	98	98	98

APPENDIX X

Effect of seed treatment on the percentage emergence of injured and non-injured wheat

Treatment	Percent daily emergence											
	1	2	3	4	5	6	7	8	9	10	11	12
Injured												
Check	0	0	26	66	78	84	86	86	88	88	88	88
Form. 1:320	0	0	0	12	26	36	40	40	40	40	42	42
Ceresan ½oz.	0	0	6	42	86	90	90	90	90	90	90	90
Nomersan 2oz.	0	0	0	48	74	78	80	82	82	82	82	82
Spergon 2oz.	0	0	12	52	80	86	86	88	88	88	88	88
Non-injured												
Check	0	0	2	64	84	86	86	86	90	90	90	90
Form. 1:320	0	0	0	2	54	70	72	72	72	72	72	72
Ceresan ½oz.	0	0	16	64	88	92	94	94	94	94	94	94
Nomersan 2oz.	0	0	0	46	72	80	86	88	88	88	88	88
Spergon 2oz.	0	0	12	78	90	92	94	94	94	94	94	94

APPENDIX XI

Effect of seed treatment on the daily root growth of injured and uninjured Victory oats after dehulling

	Daily root length in inches*											
Treatment	1	2	3	4	5	6	7	8	9	10	11	12
Injured seed												
Check	36	54	95	150	201	225	244	254	264	269	288	296
Form. 1:320	1	2	3	4	5	5	5	6	6	6	6	6
Ceresan ½ oz.	21	35	60	90	132	158	187	205	225	243	257	270
Spergon 2 oz.	42	66	116	175	235	270	295	313	344	360	380	405
Non-injured seed												
Check	35	55	95	151	209	257	283	302	331	357	375	387
Form. 1:320	4	5	8	10	13	19	21	25	27	28	30	33
Ceresan ½ oz.	48	70	115	157	248	316	374	413	454	488	523	558
Spergon 2 oz.	48	64	120	161	239	277	312	343	370	384	409	428

* Average per container

APPENDIX 12

List of chemical compounds used in these studies

Compound	Trade name	Manufacturer	Percentage active ingredient	Percentage inert ingredient	Mercury equivalent
Ethyl mercury phosphate	New improved ceresan	Bayer Semesan Co. Inc., New York, U.S.A.	5.0	95.0	3.8
Formaldehyde	Formalin	Standard Chemical Co., Montreal, Quebec	40.0 (approx.)	60.0	---
Tetrachloropara-benzoquinone	Spergon	United States Rubber Co., Naugatuck Chem. Div.	98.0	--	---
Tetramethylthiuram disulfide	Nomersan	Imperial Chemical Ind., Ltd., Blackley, Manchester	--	--	---